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Foreword

Introductory Remarks on the Current Status of Antibiotic Therapy and the Outlook for Future Developments*

THE history of antimicrobial therapy in the two decades since the discovery and introduction of sulfanilamide has been one of continuous change and rapid growth. The first half of this period saw steady improvements within the group of sulfonamide drugs and the successive introduction of new derivatives possessing increased or broadened activity, or decreased toxicity, or all of these attributes. Following the introduction of penicillin into general use, continued activity in the field of antimicrobial therapy (apart from the developments with respect to malaria) resulted chiefly from the exploitation of individual sulfonamide drugs. These were either older and previously discarded drugs exhumed for the purpose or slightly altered derivatives alleged to be superior to those already established. A few additional agents such as nitrofurantoin and antituberculous drugs were also introduced, but these have more limited applications.

The development and introduction into general therapeutic use of a succession of antibiotics with distinctive properties related to the spectra of their antimicrobial action and to their pharmacologic and toxic properties has characterized the period since penicillin became available. Although the antibiotics have now assumed a dominant role in antimicrobial therapy, it is of interest that the production of sulfonamide drugs in this country has continued at a high level even in recent years. This alone is strong evidence that the final solution of the problems of infectious diseases has not yet been achieved by currently available antibiotics.

This symposium does not cover the present status of the antimicrobial agents or even the newer aspects of antibiotic therapy. Nor has it been oriented about any central theme, except insofar as all the papers deal with current and up-to-date subjects of interest in the field. Many important and practical problems, such as the therapy of most of the specific bacterial infections, of viral, fungous or parasitic diseases, the prophylactic uses of antibiotics, the nutritional aspects as well as special applications to various branches of medicine and surgery are not specifically considered in this symposium.

There have already appeared a considerable number of books and monographs devoted to the consideration of single antibiotics, or to individual aspects such as mode of action or genetic features, or to clinical application in general or in specific fields, or merely to cataloging or summarizing various contributions in this area. Moreover, the literature on various phases has been summarized in successive volumes of the *Annual Review of Biochemistry*, an average of two reviews a year bearing on this subject has appeared in the *Annual Review of Microbiology* during the past few years and most of the recurring reviews on infectious diseases, such as those appearing in the *Annual Review of Medicine* and in the *Archives of Internal Medicine*, have devoted many of their discussions of therapy to the problems of antibiotics. Special journals devoted

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to antimicrobial agents have also become available, such as *Antibiotics and Chemotherapy* and, more recently, *Antibiotic Medicine* in this country, the *Journal of Antibiotics* in Japan, *Antibiotiki* in Russia and *Antibiotica et Chemotherapia* in Switzerland. The latter is an annual publication devoted solely to general reviews while the others contain chiefly original articles. The great bulk of the published contributions in this field, however, continue to appear in other medical and scientific journals.

A large amount of fundamental data and much that is of immediate and practical importance has been summarized by each of the contributors to this symposium. It would be presumptuous to include in this introduction extensive comments on these well documented presentations. However, a few generalizations based on these papers and some brief comments on certain practical problems not specifically covered by the various contributors may be in order.

The antibiotics have presented the chemists with an exciting and challenging field of adventure as indicated in Dr. Regna's paper. The chemists have responded by elaborating definitive structural formulas for most of the useful antibiotics and some definite though not complete data about the chemistry of most of the others. They have even succeeded in synthesizing by commercially applicable methods one of the most important, although chemically the simplest of these agents, namely, chloramphenicol. Of particular interest, however, have been the striking differences in the chemical structure of the various antibiotics and the fact that some of them have revealed novel structural configurations. Few of these details of chemical constitution could have been predicted *a priori* from the activity or pharmacologic properties of these agents or from their sources. With some exceptions, the many attempts to modify the chemical structure of naturally occurring antibiotics on the basis of preconceived notions or principles have not resulted in useful improvements in activity or in reduction in toxicity. This is in sharp contrast to the earlier developments in the sulfonamide era when essentially all of the useful agents were based on modifications of, or rather additions to, the structure of sulfanilamide. Even so, only an extremely small proportion of the thousands of sulfanilamide derivatives that were synthesized yielded useful improvements in activity or in pharmacologic properties.

To be sure, certain salts and esters of penicillin have proved useful either because their poor solubility and the slow release of active penicillin from the site of injection has compensated in part for the very rapid absorption and renal clearance of this agent, or because they incorporated antihistaminic or analgesic substances which are intended to help reduce somewhat its sensitizing properties or the pain resulting from its injection. With respect to dihydrostreptomycin, which commercially has been one of the most successful of the chemical derivatives, there is considerable doubt as to whether its widespread acceptance and use is justifiable, at least from the patients' point of view.

The marked similarity in the chemical structure of aureomycin® (chlortetracycline) and terramycin® (oxytetracycline), however, was predictable on the basis of (1) their parallel activity *in vitro* and therapeutically, (2) the almost complete cross resistance in organisms in which resistance to one or the other was induced by repeated exposures *in vitro* and (3) by the similarity of their major untoward effects. This was confirmed by the almost simultaneous solution of the structure of both agents in separate laboratories through somewhat different approaches and by the identification and subsequent preparation of tetracycline, the structure of which is common to both. Tetracycline also has essentially the same activity and pharmacologic properties as the other two analogues and shows essentially complete cross resistance with them. Differences in activity among these agents and in their untoward effects are chiefly quantitative. On the same basis some structural similarity, though not so close as with the tetracyclines, may be predicted for erythromycin and carbomycin since these two agents also have quite parallel though quantitatively different antimicrobial activity *in vitro* and show almost complete cross resistance; they do, however, have somewhat different pharmacologic properties.

The modes of action of the different antibiotics, as brought out by Umbreit, have shown no set pattern. Although certain types of activity have been described for many of the antibiotics, these had not been predicted either from their antimicrobial spectra or from their pharmacologic or toxic effects in animals or man. Indeed, that could hardly have been expected from the

diversity and novel character of their chemical structures. It is not surprising, therefore, that in spite of the plausible nature of the principle of antimetabolites or biological antagonisms and in spite of the many other known modes of action (as enumerated by Umbreit) proved or proposed for some of the antibiotics, there has been no new development of a significant new agent based on the knowledge gained from those already in use. Perhaps it is only a matter of time until sufficient data will be acquired concerning the structure, chemical composition and biological functions of microbial organisms and their host cells which will permit a better understanding of their reactions to various chemical agents. Then perhaps specific substances may be synthesized with predictable antimicrobial properties that will be useful and well tolerated as therapeutic agents.

Meanwhile, the agents being developed are for the most part the result of laborious screening programs, conducted largely by pharmaceutical manufacturers or by a relatively small number of other laboratories. Commercially, this seems to be the most feasible approach because the financial yield to be expected from the chance discovery of a single useful agent would more than adequately compensate for the large expenditure involved in these screening programs. To be sure, more scientific methods of deriving new agents are also being pursued but the agents resulting from such efforts must also be put through an essentially similar screening program to test their practical value, and the yield of useful agents thus obtained has yet to approach that derived from the crude screening methods which start with raw materials such as cultures or samples of soil.

The virus diseases offer the greatest challenge in this respect because of the complete dependence of the parasite for its multiplication on the chemical components and activities of the host cells. Although it may seem a discouraging task to find agents that would interfere differentially with the metabolism and multiplication of the virus parasite while permitting the maintenance of functional integrity of the host cell, the outlook is not entirely gloomy. Here again attacks on the problem, based on interference with specific metabolic pathways or cell-virus interactions, have yielded only results of minor importance which suggest only that such an approach may be feasible. On the other hand considerable encouragement was offered by the chance discovery that such antibiotics as chloramphenicol, chlortetracycline and oxytetracycline are effective in the prevention and treatment of experimental infections with all of the known rickettsial agents against which they have been tested, as well as against infections with organisms of the psittacosis-lymphogranuloma venereum group. Clinically, the striking effectiveness of these broad-spectrum antibiotics has been universally demonstrated in all rickettsial infections in which they have been used; this is ample evidence that the intracellular nature of the parasite need not interfere with successful chemotherapy. Demonstration that isoniazid, an effective and relatively non-toxic drug, penetrates the tubercle bacillus even when it is located intracellularly, lends further support to this view.

Unfortunately, in the treatment of virus and rickettsial infections, as in the case of antibacterial therapy, the only practical and useful agents have stemmed from broad screening programs rather than from preconceived formulations. The prospects for more rapid developments in this area have been greatly enhanced by the enormous strides recently made in the field of tissue cultures, not only in the cultivation of viruses but also in the possibilities available for learning more about the metabolic processes of both viruses and tissue cells and their interrelationships.

Meanwhile there has been considerable confusion in the literature concerning the value of various antibiotics in the treatment of diseases definitely or presumably due to viruses, namely, mumps, influenza, infectious hepatitis, infectious mononucleosis, chicken pox, herpes zoster and primary atypical pneumonia. In the case of each of these infections favorable reports are more or less balanced by equally convincing evidence of failures of the same agents to influence the same diseases. In most instances data from well controlled studies have failed to support the efficacy of any agents in these diseases. In the case of primary atypical pneumonia, even "controlled" experiments have yielded conflicting results when done at different times and by different observers; these conflicting results may be based on differences in the types of clinical material included in the different studies. The answers in this instance must await the development of more definitive methods of etiologic diagnosis. The possibility must also be considered that the different results

reported for some diseases may depend on the nature of the basic bacterial flora in the patients or on the complicating bacterial infections; these may vary from place to place and from time to time in the same hospital depending, in part, on the types and extent of antibiotics used in the particular hospital.

The genetic patterns involved in the development of resistance to antibiotics by bacteria *in vitro* are reviewed here by Bryson and Demerec. These authors also discuss the clinical and therapeutic implications of the resistance patterns. Here again we are confronted with variability in connection with the antibiotics and the bacterial species. There is as yet no clearly defined basis for predicting the resistance pattern either from the chemical structure of the antibiotic or from the nature of the microbial species. The study of the genetic patterns involved in the emergence of resistant strains during therapy is obviously not possible by the usual *in vitro* methods. The selection of naturally resistant species through the elimination of sensitive ones seems obvious. It can only be presumed, however, that increased resistance in successive isolations of organisms of the same species during therapy arises through the same mutation-selection mechanisms that are demonstrable *in vitro*. However, when markedly penicillin-resistant and penicillinase-producing variants of Micrococcus pyogenes, var. aureus (Staphylococcus aureus) appear in large numbers in patients under treatment with penicillin soon after the demonstration of highly sensitive and non-penicillinase producing strains, it cannot be stated with certainty which of the two mechanisms was involved. A number of other unsolved problems in this field are also mentioned by Bryson and Demerec in their introduction.

The mechanism whereby the incidence of resistant staphylococci has increased has been clarified to some extent by epidemiologic studies. The selection of resistant staphylococcal strains through the extensive and intensive use of antibiotics and their spread by contact among patients and hospital personnel has been demonstrated in many studies of penicillin. To some extent similar demonstrations have been made with chlortetracycline and oxytetracycline and, in one hospital, with erythromycin. The rate at which staphylococci that are highly resistant to these antibiotics replace the sensitive ones in the nasopharynx or exposed wounds, whether or not treatment with antibiotics is given, suggests that these hospitalized patients offer a particularly fertile ground for implantation and multiplication of the penicillin-resistant and penicillinase-producing staphylococci.

The situation is quite different, however, in patients under treatment for staphylococcal infections in deep foci, such as endocarditis or osteomyelitis, which have no ready access to contamination from without and where sterilization of the focus during treatment occurs very slowly. In these instances the resistance of the successive strains of staphylococci isolated from blood cultures during treatment with penicillin increases progressively and can be assumed to arise by mutation-selection. The latter instance is analogous to the emergence of tubercle bacilli of progressively increasing resistance in most patients with tuberculosis who were treated with streptomycin or isoniazid. In such cases combinations of agents each of which is effective against the pretreatment strain may have their greatest use in delaying the emergence of resistant variants while the depletion of the bacterial population at the infected focus proceeds gradually and progressively. On the other hand, when one is dealing with a mixed flora of sensitive and resistant species, for example in urinary tract infections, as brought out in the paper by Kass, or with chronic respiratory infections or open wounds, the use of multiple antimicrobial agents cannot prevent the inevitable replacement of sensitive by resistant flora. Emergence of resistant species or strains can be delayed in such instances only to the extent to which the antimicrobials used are effective against the entire flora, including the new strains which may find their way into the infected areas. In this respect the antibiotic-resistant staphylococci now prevalent in many hospitals may be considered an essentially different species from that of sensitive strains of staphylococci and may be comparable to proteus, pseudomonas or monilia.

The problems related to resistance also have important implications with respect to prophylaxis. In view of the recent experiences with antibiotic-resistant staphylococci in hospitals and with the spread of infections with sulfonamide-resistant streptococci during the extensive prophylactic use of sulfadiazine in military establishments during World War II, it must be borne in mind that even the results of controlled experiments on the prophylactic use of antimicrobials can be considered valid only

with respect to the time and place and the conditions under which such experiments are conducted. One must now take into account the broad implications of the changing flora during antimicrobial therapy of the individuals and the groups to whom the prophylaxis is applied. The individual may be rendered more vulnerable to infection by organisms which are not susceptible to the prophylactic agent and which may normally exist in symbiosis, or in a state of equilibrium with those that are susceptible and are to be eliminated by the therapy. This is wholly apart from the possibility of enhanced growth or virulence of certain species in the presence of antibiotics, a factor which has not yet been fully authenticated in human infections. The routine use of antibiotics prophylactically in large numbers of patients in hospitals is probably the major cause for the rapid increase in the incidence and pathogenic significance of antibiotic-resistant staphylococci.

In any event the prophylactic use of antimicrobial agents, particularly for long periods and in large numbers of persons, may actually increase the probability that infections when they do occur will be due to organisms resistant to just those antibiotics used in the prophylaxis. On the other hand the use of prophylaxis against highly sensitive organisms for brief periods may be highly useful and fully justified either when applied to large masses of persons, as in the mass use of sulfadiazine for one or two days in order to eliminate meningococcal carriers and to prevent the spread of meningococcal infections, or when applied to individuals in single doses, as in the use of penicillin following venereal exposure to prevent gonococcal infections. The continuous use of penicillin for long periods to prevent streptococcal infections and hence recurrences of rheumatic fever may also be justified. In this instance, however, the evidence should be reviewed from time to time in order to determine whether the risks of such continuous prophylaxis are increasing due to infections with penicillin-resistant organisms and if that should occur whether it might not be preferable to substitute prompt and adequate penicillin treatment at the beginning of the disease or following definite exposure to streptococcal infections.

The papers by Bryer and Kass call attention to the consequences of extensive, repeated or prolonged use of antimicrobial therapy in conditions in which reinfection or continued infection with antibiotic-resistant organisms present a threat to the subsequent successful use of antimicrobials. The examples of chronic urinary tract infections loom large in this respect but similar problems are present in chronic non-tuberculous bronchopulmonary suppuration with bronchiectasis and in abscesses which permit the pooling of infected secretions that are partly protected from systemic or even topical therapy. In such cases continuous therapy with small well tolerated doses over long periods has proved successful in reducing morbidity for a limited time in certain types or groups of individuals. The use of such treatment on a large scale is fraught with the same dangers as other long-term prophylaxis but the danger may be greater because larger doses are often employed and because subsequent exacerbations of infections are associated with organisms that are more resistant.

Some of these cases offer a real challenge. First, early diagnosis and treatment of the original infections set the stage for the later chronic and resistant forms. Secondly, the discovery of new methods of approach may help to determine the underlying defects which predispose to the infections and the local conditions which permit the infections to establish themselves in these areas. The successes thus far achieved in the field of chemotherapy of tuberculosis, so ably reviewed here by Ebert, and the prospects which he outlines for further progress in this disease give hope that similar successes can be expected in other chronic, non-tuberculous infections. Even in tuberculosis, however, as Hobby's review on the viability of tubercle bacilli in treated patients indicates, the goal of "cure" by complete eradication of infection has not yet been achieved.

In his contribution to this symposium von Oettingen has reviewed the reports on the large number of complications that may accompany or follow the use of antibiotics. Numerically, and from the long range point of view, the most important group of complications is that which includes the various manifestations of hypersensitivity. The very extensive use of penicillin has made this aspect particularly prominent in connection with that agent. The frequency and severity of hypersensitivity reactions to penicillin has been further enhanced by the introduction and great popularity of the repository forms. Theoretically, at least, these repository forms permit not only a greater antigenic

and sensitizing effect but may also render the patient more vulnerable to prolonged effects from a "shocking dose" when given later to previously sensitized individuals or from residual penicillin still present at the repository site in patients developing sensitivity reactions during treatment. Moreover, the possibility of inadvertent injection of these insoluble preparations into a vein may have contributed to some of the severe and fatal anaphylactoid reactions.

From this point of view, therefore, the early successes reported with the use of preparations having the most prolonged activity, such as benzathine penicillin G, should be interpreted with cautious reserve. One should be alert to the possibility of serious and prolonged reactions from later injections in patients who have received such preparations and have been sensitized by them and to the delayed reactions due to the last residues of these materials so slowly absorbed from the repository sites. It is possible, of course, that this very property of slow absorption may in some instances serve to "desensitize" the patient. If so, this would be a very welcome and salutary effect for which there is little in the way of authentic precedent. At any rate, more data carefully collected and documented are needed before one can feel fully hopeful about the general use of such products. Caution is advised especially in persons who are prone to hypersensitivity reactions.

The possibility that the so-called collagen diseases, particularly periarteritis nodosa and disseminated lupus erythematosus, may result from reactions to antibiotics has been suggested from some case reports. The demonstration of the transient appearance of "L.E." cells during penicillin reactions lends support to this possibility but cannot yet be accepted as proof. Such suggestions have already been made in the past about sulfonamide therapy.

The high rate of sensitization from the more commonly used antibiotics and the sulfonamides when used topically, particularly in ointments, is noteworthy although it is generally not given adequate serious consideration except by allergists. For topical therapy it may be preferable to use those antibiotics which, because of their nephrotoxicity or neurotoxicity, are less commonly used by injection, namely, bacitracin, polymyxin and neomycin. Fortunately these antibiotics appear to produce sensitization from topical application rather infrequently, although adequate data on this aspect are not available. Even so, the prolonged use of these agents locally, especially in ointment form, is not to be encouraged because sensitization may occur in some individuals. Their use for specific purposes and for relatively short periods in the form of solutions may be particularly advantageous in superficial wound infections, or in walled-off and localized abscesses, or in infections of body cavities due to susceptible organisms. The three aforementioned antibiotics may also be used orally for infections within the intestinal tract or in preparation for bowel surgery since they are poorly absorbed by this route.

It is apparent that almost all organ systems may be involved to varying degrees and with varying specificity in the complications of antibiotic therapy. Involvement of the blood and blood-forming organs has come into prominence with chloramphenicol and in rare instances with streptomycin, and they have been implicated as the likely causes of some cases of aplastic anemia. Although the toxicity of chloramphenicol for the blood has been related to the nitrobenzene portion of the molecule, it is noteworthy that the substituted amine portion has certain similarities to the structure of the nitrogen mustards. Fortunately, aplastic anemia is a relatively rare complication so that it need not deter one from using these agents in the treatment of serious infections whenever particularly indicated or advantageous. It is well to recall that agranulocytosis is an important feature of prolonged therapy with any of the sulfonamide drugs and that severe hemolytic anemia is the most striking and serious complication of sulfanilamide therapy.

Gastrointestinal complications have also assumed special significance recently. Nausea, vomiting and diarrhea have been a feature of oral therapy with most of the antibiotics, varying considerably with the different agents and with the dosage used. These had not been considered to be serious. However, the simultaneous spread of antibiotic-resistant and enterotoxic strains of staphylococci in some hospitals has resulted in the occurrence of severe staphylococcal diarrheas and has brought into prominence the association of such staphylococci with so-called pseudomembranous enterocolitis, the latter occurring particularly as a complication of bowel surgery in patients who have received antibiotics pre- and postoperatively. These complications appear to be part of the

general effect of the extensive use of antibiotics in hospitals, especially of the almost universal practice of giving them for prophylaxis pre- and postoperatively. They may also be considered part of the general problem of superinfections with resistant bacteria during antibiotic therapy.

The relation of some of these untoward reactions to vitamin deficiencies is strongly suggested by the symptomatology in many patients. This has stimulated the use of vitamin mixtures in large amounts during antibiotic therapy for the prevention and therapy of such symptoms. Some of the recommendations for the use of vitamins during antimicrobial therapy are based on experimental evidence derived from laboratory animals placed on purified diets after which specific deficiencies are produced, then cured or prevented by incorporation of the appropriate vitamins. The evidence for such deficiencies in man, however, is incomplete except in very special instances, and the significance of some of them is not clear. Thus, although increased riboflavin excretion accompanies the nitrogen deficit occurring during administration of chlortetracycline or oxytetracycline, the administration of large doses of riboflavin, alone or with other vitamins, has not been clearly shown to prevent or to relieve the untoward effects of these antibiotics. On the other hand the administration of pyridoxine, to replace the excess excreted, appears to have a salutary effect in preventing or relieving the neurologic complications of isoniazid therapy.

It is not possible to predict the type, frequency or severity of the complications resulting from any of the antibiotics from their antimicrobial spectra, mode of action or chemical structure. Many toxic clinical manifestations could not even be anticipated from toxicity and pharmacologic studies in laboratory animals. While the mechanisms of some of the toxic effects have been worked out, most of them still remain unexplained. Failure to anticipate untoward biological effects from the chemical structure was also true of sulfonamide drugs; each new sulfanilamide derivative, while altering antibacterial activity to a slight extent, introduced greater differences in toxic effects, of which only those related to solubility could readily have been predicted. Almost all of the information concerning toxicity of the antimicrobial agents and significance of their untoward effects has become available only as a result of clinical experience.

It has been repeatedly indicated here that the discovery of antibiotics and most of our knowledge concerning their actions and usefulness have been arrived at empirically. It should also be emphasized that most of what is accepted as good practice in the use of antimicrobials, particularly as to the relative merits of different agents and dosages and their value in prophylaxis and therapy, is based on studies that are not adequately controlled. Thus some of the recent reports on the superiority of benzathine penicillin G over other forms of prophylaxis in the prevention of rheumatic fever may appear superficially to be quite convincing but cannot be accepted without reservation because of the failure to include adequate controls. Comparisons with similar observations made in other clinics, even during the same period, or with material collected previously or subsequently in the same clinic may not be altogether valid because the prevalence of streptococcal infections or the opportunities for acquiring such infections in the two given groups may be entirely different. Thus in a recent study on contagious diseases carried out over many months in one large hospital, pneumococci or hemolytic streptococci were rarely encountered in any patients either at the time of admission or as new organisms in the respiratory flora of tracheotomized poliomyelitis patients. When those organisms were encountered they were rapidly eliminated.

There are certain notable exceptions, among which are the extensive and carefully controlled studies on the treatment of streptococcal infection. Particularly worthy of mention are the contributions on the chemotherapy of tuberculosis resulting from the cooperative studies of the Veterans Administration, Army and Navy Hospitals. In these studies, carried out continuously over the last few years, many of the most important practical details of the chemotherapy of tuberculosis have been worked out in human subjects with a critique worthy of emulation by all groups of investigators interested in clinical therapeutics.

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Symposium on Newer Aspects of Antibiotics

Chemistry of Antibiotics of Clinical Importance*

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THE term "antibiotic," first introduced by Waksman[1] in 1942, has more recently been amplified[2] to designate any chemical substance produced by a microorganism which has the capacity to inhibit the growth of bacteria and other microorganisms or to destroy them. Although certain arbitrary limitations are implicit in this definition, the present review will be held within the boundaries of this concept and will be further limited to a discussion of those antibiotics which are in current use in the treatment of many types of human infections.[3]

Use of the term "antibiosis" to designate anti-living processes has been traced to Vuillemin[4] who in 1889 referred to the phenomenon of "one creature destroying the life of another in order to sustain its own—one being in unrestricted opposition to the life of the other." The first association of "antibiosis" with disease-producing organisms is credited to Pasteur and Joubert[5] who in 1877 reported that certain aerobic non-pathogenic bacteria interfered with the growth of anthrax bacilli. While numerous reports dealing with antibacterial action were published during the subsequent years, the use of "chemotherapeutic agents" in the treatment of bacterial infections was, until comparatively recently, largely disappointing. This course, however, was altered by the discovery of sulfanilamide by Tréfouël and co-workers[6] in 1935 and with the notable discovery made in 1929 by Fleming[7] who detected the lytic action of an accidental mold contaminant on a plate which had previously been seeded with staphylococci. On cultivating this mold—which was later identified as a strain of Penicillium notatum—he found that the broth had a marked inhibitory effect on certain gram-positive pathogenic organisms. He designated the active principle "penicillin." This work was extended by Clutterbuck, Lovell and Raistrick[8] who in 1932 showed that penicillin, produced by the mold on a synthetic medium, was a labile substance and would retain its biologic activity only at neutrality.

Stimulated by the work of Dubos[9] on gramicidin, and that of Colebrook and Kenny[10] on the effectiveness of Prontosil® in the treatment of puerperal infections, Chain, Florey and their co-workers[11] subsequently undertook a study of antibacterial substances, in particular of penicillin. In 1940 methods were developed for the recovery of adequate amounts of penicillin in the form of a brown powder, and it was shown that injections of penicillin could control experimental infections in mice, due to hemolytic streptococci, staphylococci and pathogenic anaerobes.[11] The first clinical trials in humans were reported in 1941 by Florey and his associates[12] who showed that penicillin was therapeutically active in remarkably high dilutions, and that it was not inactivated by blood, pus or tissue.

Following this publication on the properties of penicillin, marked changes took place in the field of chemotherapy and infectious diseases. Since 1940 innumerable substances produced by bacteria, actinomycetes, fungi, algae, lichens, higher plants, animals and man have been studied. Most antibiotic agents, however, have failed to fulfill one or more of the criteria of a

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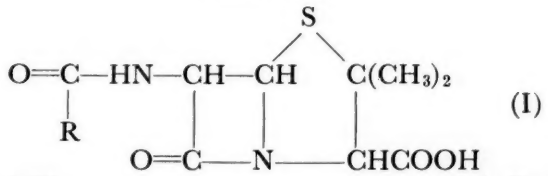
satisfactory chemotherapeutic agent, and thus have never become useful adjuncts of modern medicine.

PENICILLIN

In addition to mold pigments, the original strain used for the production of penicillin produced at least six closely related penicillins. (Table I.)

TABLE I

PENICILLINS



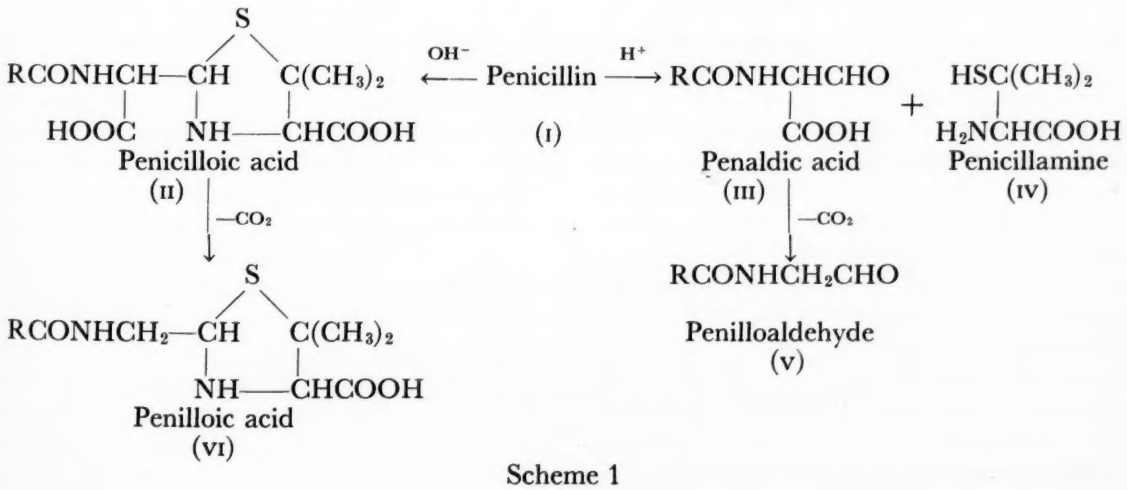
(I)

Penicillin		*Side Chain* R
(G)	Benzyl	$C_6H_5CH_2-$
(X)	p-Hydroxybenzyl	$HOC_6H_4CH_2-$
(F)	2-Pentenyl	$CH_3CH_2CH{=}CHCH_2-$
	3-Pentenyl	$CH_3CH{=}CHCH_2CH_2-$
(Dihydro F)	n-Amyl	$CH_3CH_2CH_2CH_2CH_2-$
(K)	n-Heptyl	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2-$

The penicillins produced in media free of precursors differ only with respect to the side chains R, which are attached to a common nucleus (I). All of these penicillins are strongly optically active. They are monocarboxylic acids of about pK 2.8 which readily decarboxylate into biologically inactive derivatives. Benzylpenicillin (G) is the chief product of modern fermentation methods and the most important marketed form of the antibiotic. One mg. of the crystalline sodium salt is equal to 1,667 Oxford units.

The chemical structure of penicillin was the subject of an intensive Anglo-American collaborative study during World War II. These investigations resulted in a series of degradation reactions which were extremely difficult to interpret and led to the proposal of several structural formulas for the antibiotic. Eventually, attention was focused particularly on the oxazolone-thiazolidine and β-lactam structural variants. Conclusive support for the β-lactam structure (I) was provided by infrared absorption and crystallographic x-ray studies on crystals of the rubidium and potassium salts of benzylpenicillin.[13]

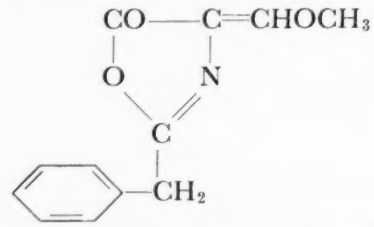
In acid solution penicillin (Scheme 1) is readily cleaved into an amino acid, penicillamine (IV) (β-thiol-*D*-valine) common to all penicillins, and a second fragment penaldic acid (III) which differs with the nature of the alkyl group R of the particular penicillin. The penaldic



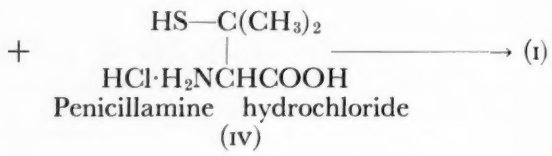
Scheme 1

acids are unstable and readily lose carbon dioxide to give the corresponding penilloaldehydes (v). Alkaline hydrolysis, on the other hand, opens the β-lactam ring of penicillin, yielding penicilloic acid (ii). The penicilloic acids readily lose carbon dioxide to form penilloic acids (vi). Acid hydrolysis also cleaves penicilloic acids (ii) into penicillamine (iv) and penaldic acids (iii).[14]

Many attempts have been made to synthesize penicillin but all have produced disappointing results. One synthetic approach, originally devised by Folkers and his co-workers and later refined by du Vigneaud and his collaborators, involved the condensation of *D*-penicillamine (iv) with 2-benzyl-4-methoxymethylene-5-(4) oxazolone (vii) (Scheme 2) to yield a mixture containing 0.1 per cent of penicillin activity.[15] The presence of benzylpenicillin was established unequivocally by isolating the antibiotic from the reaction mixture by Craig[16] countercurrent distribution.



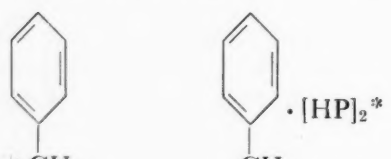
2-Benzyl-4-methoxymethylene-5(4) oxazolone
(vii)



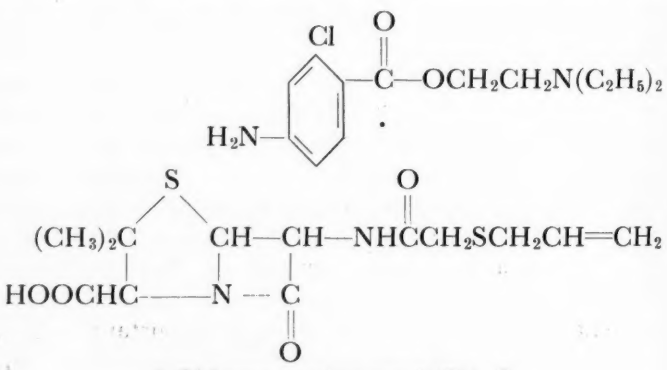
Penicillamine hydrochloride
(iv)

Scheme 2

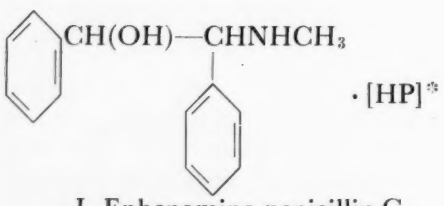
The instability of penicillin greatly hampers the organic chemist in his efforts to vary the



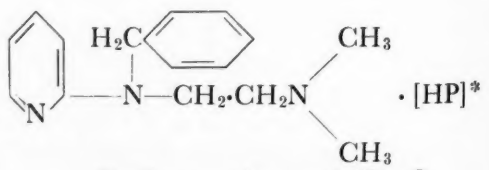
N,N′-Dibenzylethylenediamine dipenicillin G
(viii)



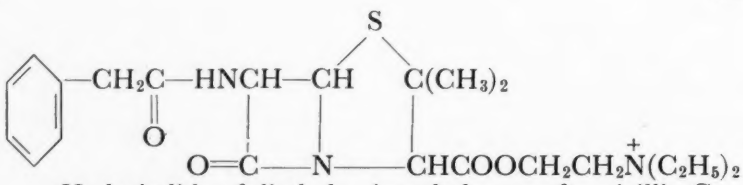
2-Chloroprocaine penicillin O
(ix)



L-Ephenamine penicillin G
(x)



Pyribenzamine penicillin G
(xii)



Hydroiodide of diethylaminoethyl ester of penicillin G
(xi)

* [HP] represents the acidic form of penicillin.

molecule. For this reason a considerable number of biosynthetic penicillins[17,18] have been prepared by fermentation in the presence of substituted amides whose acyl groups are incorporated into the penicillin molecule. This process is limited to alterations of the R-group of (I) and does not vary the main structural features of the penicillin molecule. Further modifications are limited to (1) the R-groups in those biosynthesized penicillins with functional groups, such as the OH group in *p*-hydroxybenzylpenicillin, (2) the preparation of esters formed by reacting diazoalkanes with the free acid of penicillin and (3) formation of the amide.[19] The organic chemist has been more successful in devising a series of penicillin compounds which have low solubility in water or body fluids, decreased allergenic reactions and reduced pain at the site of injection.

While the procaine salt[20,21] continues to be the most widely used repository preparation of penicillin G, several other salts of penicillin are finding application in current medical practice,[3] viz., N,N′-dibenzylethylenediamine dipenicillin G (benzathine penicillin G) (VIII)[22] 2-chloroprocaine allylmercaptomethyl penicillin (chloroprocaine penicillin O) (IX);[23,24] *L*-N-methyl-1,2-diphenyl-2-hydroxyethylamine penicillin G (*L*-ephenamine penicillin G) (X);[25] the hydroiodide of diethylaminoethyl ester of penicillin G (Neopenil) (XI);[26] the N,N-dimethyl-N′-benzyl-N′-(α-pyridyl) ethylenediamine penicillin G (pyribenzamine penicillin) (XII);[27] and dibenzylamine penicillin G.[28]

STREPTOMYCIN

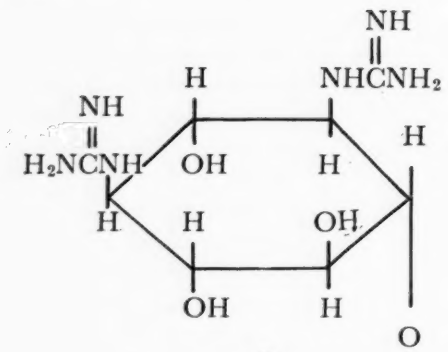
The remarkable antibacterial properties of penicillin stimulated the search for other biologically produced antimicrobial agents. As a result of one such program the isolation of streptomycin from two cultures of streptomycin-producing strains of Streptomyces griseus was reported by Waksman and his group[29] in 1944. The crude antibiotic was found to be active against certain gram-positive and gram-negative bacteria,[29] and active against the mycobacteria, particularly human pathogens.[30]

Almost immediately several groups of investigators undertook the purification of the antibiotic.[31–34] However, even before pure crystalline streptomycin was available the effectiveness of crude material had been demonstrated in counteracting disease processes, in experimental animals, due to Pasteurella tularensis,[35] Salmonella schottmülleri,[36] S. pullorum,[37] Mycobacterium tuberculosis,[38] etc., and had also been studied in the treatment of human tuberculosis.[39]

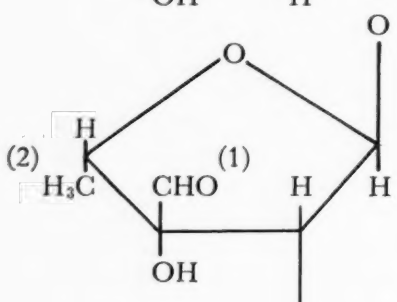
Streptomycin is an optically active, triacidic base, with the molecular composition $C_{21}H_{39}N_7O_{12}$. The antibiotic forms various salts of which the sulfate, $2(C_{21}H_{39}N_7O_{12})\cdot 3H_2SO_4$, the trihydrochloride and calcium chloride double salt, $2(C_{21}H_{39}N_7O_{12}\cdot 3HCl)\cdot CaCl_2$, are of commercial importance. The crystalline calcium chloride double salt is of great aid in eliminating impurities in crude streptomycin concentrates.[40] These salts of streptomycin are characterized by their ready solubility in water. The base is insoluble in chloroform, ether, etc., but is soluble in water and alcohol. The hydrochloride salt is quite soluble in methyl alcohol in contrast to the sulfate which is obtained by precipitation from methyl alcohol solutions.

The very difficult task of determining the structure of streptomycin was carried out principally by Folkers, Wintersteiner, Carter, Wolfrom and their respective co-workers. These studies have been reviewed several times.[41–43] Streptomycin (XIII) is composed of three moieties (Scheme 3): streptidine (XIV), streptose (XV) and N-methyl-*L*-glucosamine (XVI) joined together by glycosidic linkages.[44] In acids the weaker glycosidic bond in streptomycin,[44,45] between streptidine and streptose, is hydrolyzed to give streptobiosamine (XVII)[46–48] and a *meso* form of 1,3-diguanido-2, 4,5,6-tetrahydrocyclohexane (XIV),[49–51] whose structure has been proved by synthesis.[52,53] The basic disaccharide-like substance (XVII), can be degraded further by acid hydrolysis to N-methyl-*L*-glucosamine,[54] the non-natural form of this amino sugar, the structure of which has been established by synthesis.[55] Because of its instability, the determination of the structure of the streptose moiety presented considerable difficulties but was finally shown to be 3-C-formyl-5-deoxy-*L*-lyxose (XV), a pentose containing the reactive aldehyde group associated with the streptomycin molecule.[47,48,56–59]

The presence of this aldehyde group was demonstrated in streptomycin by oxidation with bromine to an acid, and by the formation of oxime and semicarbazone derivatives, all of which are biologically inactive.[46] Reduction of the aldehyde to an alcohol group, however, by hydrogenation with platinum[60] or nickel[61] catalysts gives dihydrostreptomycin (XVIII). Dihydrostreptomycin possesses a biologic ac-



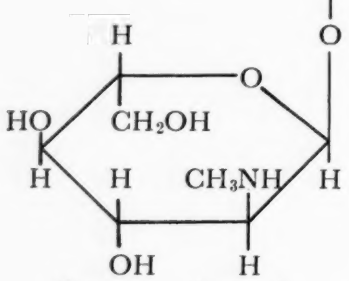
Streptidine (xiv)



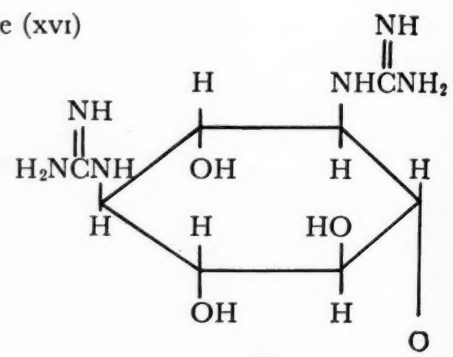
Streptose (xv)

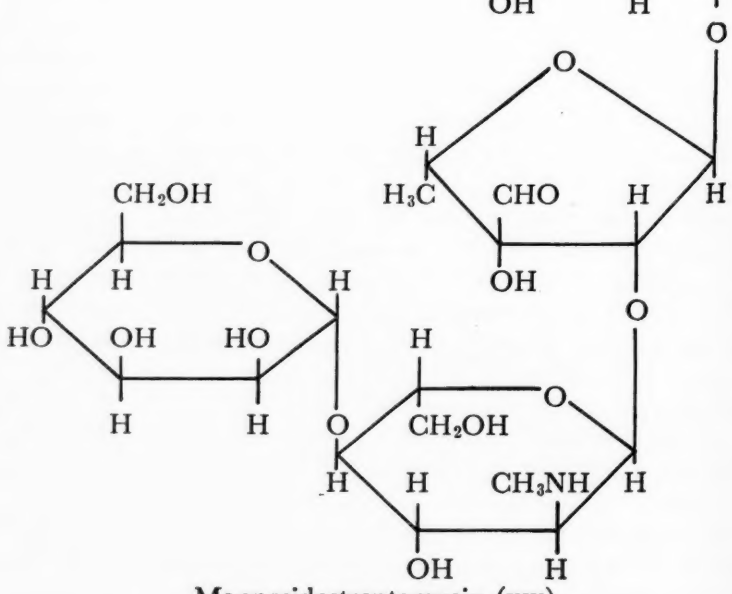
Streptobiosamine (xvii)

N-Methyl-L-glucosamine (xvi)



Streptomycin (xiii)





Mannosidostreptomycin (xix)

(1) CHO → CH_2OH=Dihydrostreptomycin (xviii)
(2) CH_3 → CH_2OH=Hydroxystreptomycin (xx)

Scheme 3

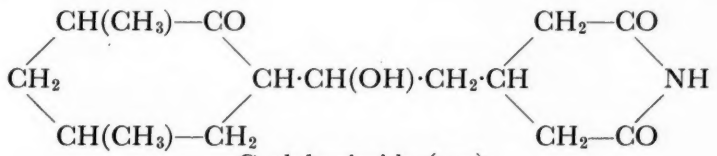
tivity comparable to streptomycin, does not respond to carbonyl reagents, does not form a calcium chloride double salt and, unlike streptomycin, is relatively stable to alkali[60] and can be crystallized as the base.[62]

The acute toxicity in mice of purified streptomycin calcium chloride complex, in terms of the LD_{50} in mg. of base per kg. of body weight, is 200 mg. by the intravenous route, above 700 mg. by subcutaneous injection and 9,000 mg. by the oral route.[63] While the acute toxicity of streptomycin is low, prolonged administration produces certain toxic manifestations.[64] In an effort to determine the contribution, if any, of minute quantities of impurities to the toxicity of streptomycin, an interesting substance was isolated from crude preparations which proved to be lethal to mice. Characterization, structure determination and synthesis of the substance showed that ammonia, under certain conditions, unites with two molecules of the antibiotic to form bis-(α-hydroxystreptomycyl)-amine which when injected into mice exhibits a toxicity 100 times greater than that of pure streptomycin.[65]

biologic activity of streptomycin. It consists of a streptomycin moiety attached glycosidically to *D*-mannose through C-4 of the N-methyl-*L*-glucosamine fragment.[73] It can be hydrogenated under conditions used for streptomycin to yield dihydromannosidostreptomycin.[69]

Hydroxystreptomycin (XX) is very closely related to streptomycin. It differs structurally only with respect to the replacement of a hydrogen atom by an oxygen atom in the streptose portion of the molecule.[74] While the biologic activity of hydroxystreptomycin is comparable to that of streptomycin, it exhibits rather high ototoxicity.[75]

Cycloheximide (XXI) β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxethyl] glutarimide, differs markedly from streptomycin in biologic activity and in chemical structure.[76,77] This antibiotic has no activity against bacteria but is quite effective against several yeasts and fungi. In concentrations as low as 0.0002 mg. per ml., cycloheximide inhibits the *in vitro* growth of the fungal pathogen, Cryptococcus neoformans, responsible for the fatal disease cryptococcosis.[78] Toxicity tests indicate an LD_{50} of approximately



Cycloheximide (XXI)

The biochemical pathways which lead to the biosynthesis, by S. griseus, of streptomycin and its closely related products still challenge investigators. Despite the complexity of this problem, some recent studies concerned with the growth requirement of this microorganism in the presence of $C^{14}O_2$ have shown that the carbon of the guanidine side chains in streptomycin was derived essentially from the carbon dioxide which was supplied.[66]

Mannosidostreptomycin, Hydroxystreptomycin and Actidione. In addition to streptomycin, several other active substances have been isolated from cultures of S. griseus. While these antibiotics are not useful clinically, those of interest because of particular chemical structures are mannosidostreptomycin (XIX) (streptomycin B),[67–69] and cycloheximide (XXI) (Actidione).[70] In addition, a substance closely related to streptomycin, hydroxystreptomycin (XX), has been isolated from various species of *Streptomyces*.[71,72]

Mannosidostreptomycin (XIX) is a streptomycin-like substance possessing about one-fourth the

150 mg. per kg. when administered intravenously in mice but the results vary with the species of animals employed.[79] Solutions of the antibiotic are highly repellent to rats even in extreme dilutions. The antibiotic crystallizes in colorless plates, m.p. 115–116°C.; $[\alpha]_D^{25} - 2.8°$ (in methyl alcohol). The simultaneous biochemical synthesis of streptomycin and cycloheximide is an example of the well recognized ability of many microorganisms to produce two or more substances with entirely distinct chemical and antimicrobial properties.

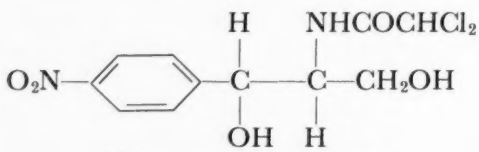
CHLORAMPHENICOL

The discovery of chloramphenicol (Chloromycetin®) by Burkholder in 1947 established the fact that an antibiotic effective against a wide variety of microorganisms could be produced by microorganisms isolated from natural sources. Such substances are now known as "broad spectrum" antibiotics. Chloramphenicol was originally obtained from a soil sample collected in a field in Venezuela[81,82] and from a

similar organism isolated from a compost soil in Illinois.[83] The original microorganism was named Streptomyces venezuelae. However, other strains of actinomycete also produce the antibiotic.[84]

The antibiotic exerts inhibitory activity toward gram-positive and gram-negative cocci and bacilli, spirochetes, actinomycetes, several rickettsiae and certain larger viruses.[81,85–88] Fungi, protozoa and smaller viruses are unaffected by the action of the antibiotic.[88] Chloramphenicol was the first antibiotic found to exert an influence on rickettsial infections such as typhus fever, and was the first antibiotic shown to be effective in the treatment of typhoid fever in humans, even though it possesses a low order of protection in experimental typhoid infections in mice.[89] This observation emphasizes the uncertainties in translating laboratory data obtained in animals to the treatment of infections in humans.

Chloramphenicol, *D*(-)-threo-2-dichloroacetamido-1-*p*-nitrophenyl-1,3-propanediol, has been found to have the structure (XXII) by a series of chemical studies, degradation reactions, physical data[90] and, finally, by synthesis.[91]



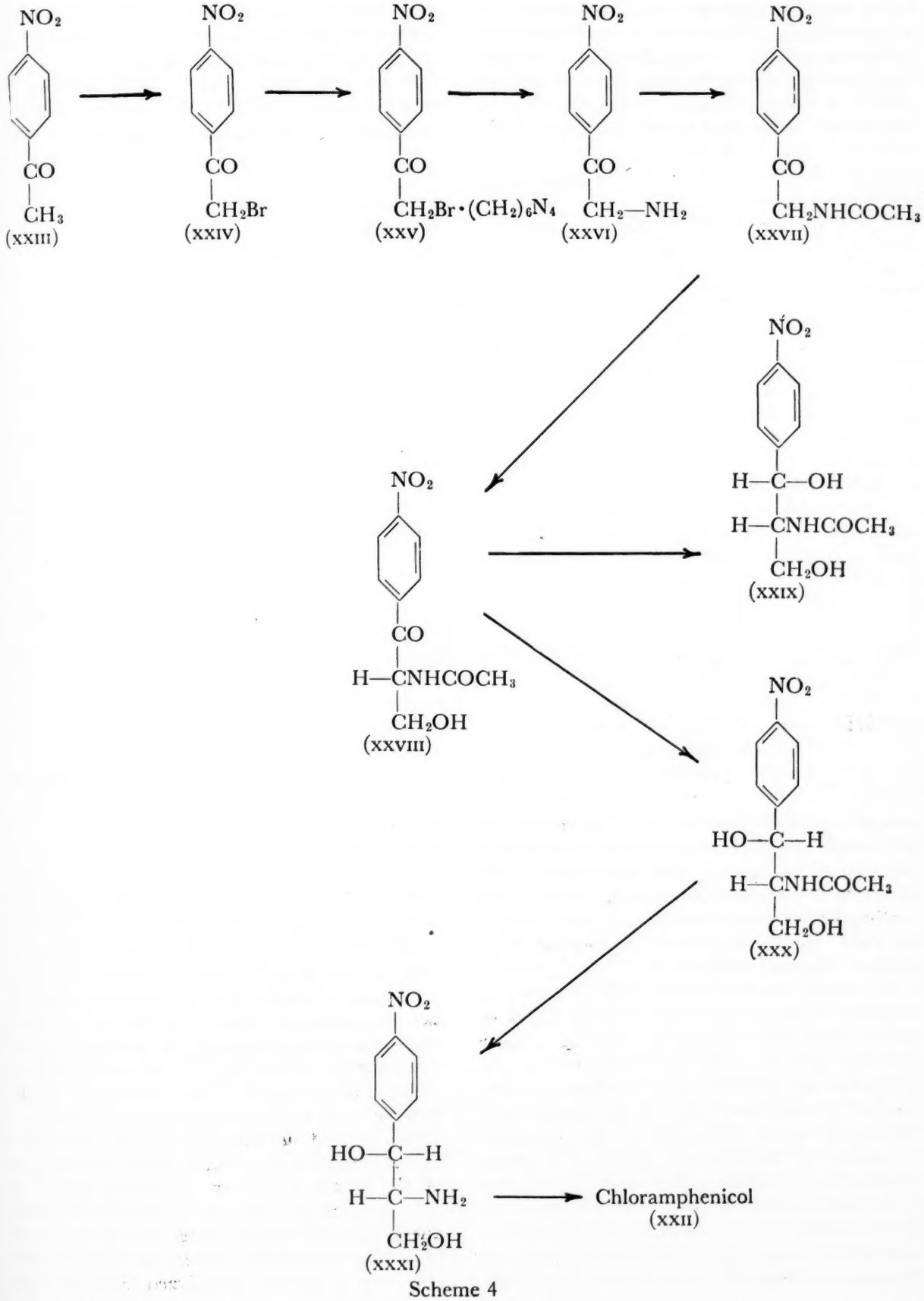
Chloramphenicol (XXII)

The structure (XXII) is related to *L*-pseudo-norephedrine. The molecule consists of a 2-acylamidopropanediol side chain containing two asymmetric carbon atoms attached to a *p*-nitrophenyl group. The first suggestions that the antibiotic contained a *p*-nitrobenzene moiety were accepted with considerable reservation because nitro groups had never been found in nature associated with living processes.[92]

Acid or alkaline hydrolysis of the antibiotic yields dichloroacetic acid and a base, $C_9H_{12}N_2O_4$, which was reconverted into chloramphenicol by reacting it with the methyl ester of dichloroacetic acid.[90] Once the method of combining these two inactive fragments into the biologically active drug was established, steps were taken to confirm the deduced constitution of the antibiotic by synthetic methods. In the first successful attempt, benzaldehyde was condensed with β-nitroethanol to yield a product which, after a series of further reactions, gave a substance identical in chemical and biological properties with natural chloramphenicol.[91] During this synthesis four isomers related to structure (XXII) are produced. Three of these isomers are essentially inactive in biologic tests. The stereochemical configuration of the side chain is specific for antimicrobial action because, of the four possible stereoisomers, only the *D*-threo possesses this activity.[90]

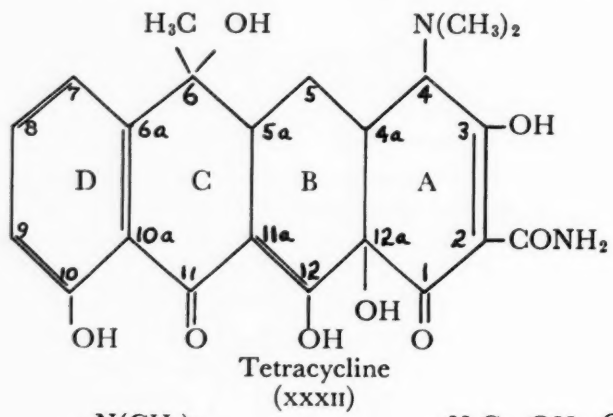
Chloramphenicol has the further distinction of being the first antibiotic to be synthesized on a commercial scale. The synthetic process in more general use is based upon a series of reactions starting with *p*-nitro-acetophenone.[93,94] Although the over-all yield is only 6 per cent, the commercial manufacture of synthetic chloramphenicol by the method shown in Scheme 4 is competitive with fermentation methods.[94] A consideration of the method of synthesis will emphasize more clearly the stereochemistry of the antibiotic.

The method can utilize acetophenone alternatively,[95] but usually *p*-nitroacetophenone (XXIII) is brominated to the ω-bromo derivative (XXIV). On treating (XXIV) with hexamethylenetetramine, the complex (XXV) is formed which on hydrolysis in alcoholic hydrochloric acid yields *p*-nitro-α-aminoacetophenone (XXVI) as a stable hydrochloride. The substance (XXVI) is unstable in the free state because it contains an amino-ketone; therefore, before proceeding to the next step (XXVI) is acetylated with acetic anhydride in order to protect the amino group. Hydroxymethylation of the acetylated product (XXVII) with formaldehyde in the presence of sodium bicarbonate yields *p*-nitro-α-acetamido-β-hydroxypropiophenone (XXVIII). The compound (XXVIII) contains an asymmetric center and thus occurs in optically active forms; however, synthetic methods produce only racemic mixtures containing equal amounts of both stereoisomers. Reduction of (XXVIII) with aluminum isopropoxide by the method of Meerwein-Ponndorf yields (XXIX) and (XXX) which now each contain two asymmetric centers, thus providing two racemic mixtures, or four compounds in all. One set of isomers represented by (XXIX) is composed of the *D*- and *L*-erythro-2-acetamido-1-*p*-nitrophenyl-1,3 propanediol disasteroisomers and the second set represented by (XXX) is the *DL*-threo racemate. The advantage of this synthetic method resides in almost exclusive formation of the *DL*-threo racemate and its ease of isolation from the complex mixture.[90] The racemate (XXX) is hydro-

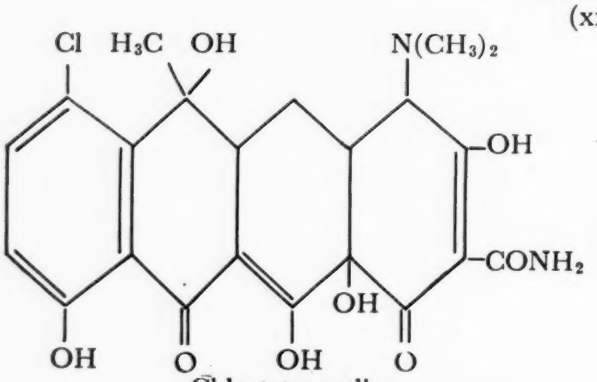

NO2
CO
CH3
(XXIII)
CH2Br
(XXIV)
CH2Br·(CH2)6N4
(XXV)
CH2—NH2
(XXVI)
CH2NHCOCH3
(XXVII)
H—C—OH
H—CNHCOCH3
CH2OH
(XXIX)
H—CNHCOCH3
CH2OH
(XXVIII)
HO—C—H
H—CNHCOCH3
CH2OH
(XXX)
HO—C—H
H—C—NH2
CH2OH
(XXXI)
Chloramphenicol
(XXII)

Scheme 4

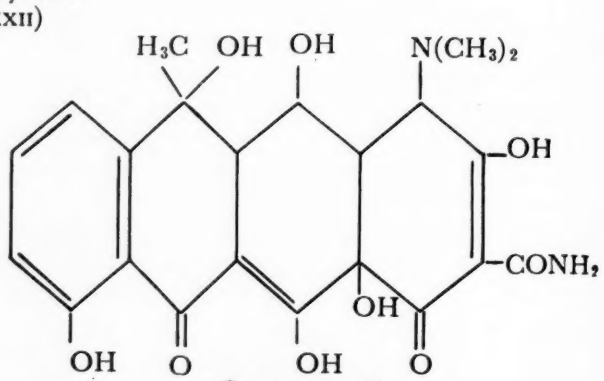
lyzed with hydrochloric acid to remove the acetyl group and the product is resolved into its optical antipodes by crystallization of its salt with an optically active acid such as tartaric acid or *D*-camphorsulfonic acid.[91] The *D*-threo salt of the latter acid forms the least soluble component of the mixture. After separation the salt is regenerated into the base (XXXI) by adding ammonium hydroxide. The final step in the synthesis is achieved by reacting (XXXI) with methyl dichloroacetate at elevated temperatures to yield (XXII), the antibiotic identical with natural chloramphenicol.[86,90]



Tetracycline
(XXXII)



Chlortetracycline
(XXXIII)



Oxytetracycline
(XXXIV)

Crystalline chloramphenicol is prepared as colorless needles, m.p. 150.5–151.5°C.; $[\alpha]_D^{25}$ + 19° (in ethyl alcohol); solubility at 25°C. in water 2.5 mg. per ml. and 150.8 mg. per ml. in propylene glycol. It is soluble in alcohols, fairly soluble in ether, and insoluble in benzene and petroleum ether. Solutions of the antibiotic in water are faintly acid (pH 5.5).[82] These solutions are sensitive to light or high temperature and decompose with the formation of hydrochloric acid.[96] Solutions at 37°C. deteriorate slowly; they have a half life of about six months. At about 5°C. there is little loss in solution during a period of two years. The dry solid is stable at ordinary temperatures and in diffuse light for at least five years.

Many ingenious analogs and structurally related compounds of chloramphenicol have been prepared and studied for their antibacterial properties. The nitro group in the molecule has been replaced by a large variety of substituents, and the dichloroacetamido group has been extensively modified. Much of this work indicates that changes in the aliphatic portion of the antibiotic molecule bring about substantial reduction or complete loss of activity. Modifications in the aromatic ring, on the other hand, do not produce such drastic results but in most cases provide some activity. An analog in which the hydroxyl group on C-1 of the antibiotic (XXII) was replaced by a keto group was found to have activity against Candida albicans.[97]

An interesting derivative and an advantageous dosage form is obtained by esterification of the terminal primary hydroxyl group in chloramphenicol with palmitic acid.[98] While the antibiotic itself is quite bitter, chloramphenicol palmitate[99] is tasteless. The ester is inactive *in vitro* but is slowly hydrolyzed enzymatically

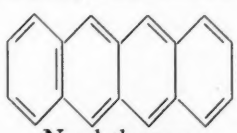
in the duodenum with release and absorption of chloramphenicol from the intestinal tract, thus providing prolonged therapeutic blood levels.

Human subjects excrete more than half of an oral dose in the urine within eight hours and up to about 80 per cent within twenty-four hours. About 5 per cent of the excreted material is unchanged antibiotic and a small amount is present in the form of hydrolytic products. More than 90 per cent, however, is in the form of a water-soluble conjugate with glucuronic acid. The glucuronide-antibiotic conjugate can be hydrolyzed enzymatically by β-glucuronidase or by alkali to regenerate free chloramphenicol.[100] Because of the presence of the nitro group in the molecule the toxicity of choramphenicol was examined critically.[92] The antibiotic was found to be of relatively low toxicity in most experimental animals but in man has been responsible for certain disorders of the blood.[101] In albino mice the maximum tolerated single intravenous dose LD_0 is 125 mg. per kg. and 1,500 mg. per kg. by the oral route. Another group of these animals tolerated 425 mg. per kg. per day orally for four weeks.[102]

It has been suggested that chloramphenicol is a phenylalanine antagonist when Escherichia coli is the test organism.[103] Interestingly, such compounds as *p*-nitrobenzaldehyde and substances which can be readily converted to *p*-nitrobenzaldehyde were found to be highly active in reversing the effect of the antibiotic on E. coli.[104]

OXYTETRACYCLINE, CHLORTETRACYCLINE AND TETRACYCLINE

Tetracycline (XXXII), chlortetracycline (XXXIII) and oxytetracycline (XXXIV) are a group of amphoteric crystalline antibiotics which contain a common hydronaphthacene skeleton. Reduc-



Naphthacene
(XXXV)

tion of these antibiotics with zinc and acetic acid successively removes the dimethylamino and C-12a hydroxyl groups. When the final reduction product is dehydrated with acid and subsequently distilled with zinc dust, naphthacene (XXXV) is obtained, thus establishing a linear tetracyclic structure for this series of substances.

Tetracycline,[105–107] the last of this series of antibiotics to be announced, is structurally related to chlortetracycline[108] and oxytetracycline.[109] Tetracycline (XXXII) is 4-dimethylamino-1,4,4a,5,5a,6,11, 12a-octahydro- 3,6,10, 12,12a-pentahydroxy- 6-methyl-1, 11-dioxo-2-naphthacenecarboxamide. Chlortetracycline (XXXIII) is the 7-chloro, and oxytetracycline (XXXIV) is the 5-hydroxy analog of tetracycline. Despite the close structural similarities of the antibiotics they differ in certain properties such as their infrared spectra, their paper chromatographic patterns and by distinct colors developed with concentrated sulfuric acid. In addition, the antibiotics of this group possess certain distinguishing chemical features since each undergoes a series of characteristic degradation reactions. The determination of the structural features of these antibiotics was spearheaded by the work on oxytetracycline.[105,110] Degradation studies were carried out predominantly by reactions involving hydrolysis, reduction and oxidation. Interpretations of the hydrolytic reactions in acid and base were particularly helpful in providing information which led eventually to elucidation of the total structure of these complex molecules.

As a group, oxytetracycline, chlortetracycline and tetracycline are soluble in glycol ethers, pyridine and dilute acid and alkali, very slightly soluble in water and in lower molecular weight alcohols, and insoluble in ether and hydrocarbons. These antibiotics readily form salts with strong acids and bases. The acid salts are well formed crystalline compounds with high solubilities in water but unless excess acid is present the crystalline amphoteric antibiotics separate on standing. Various especially interesting complex salts with calcium, magnesium, aluminum, iron, etc., have been prepared and studied for their chemical and antimicrobial properties. The salts of these antibiotics darken slightly when exposed to strong direct sunlight.

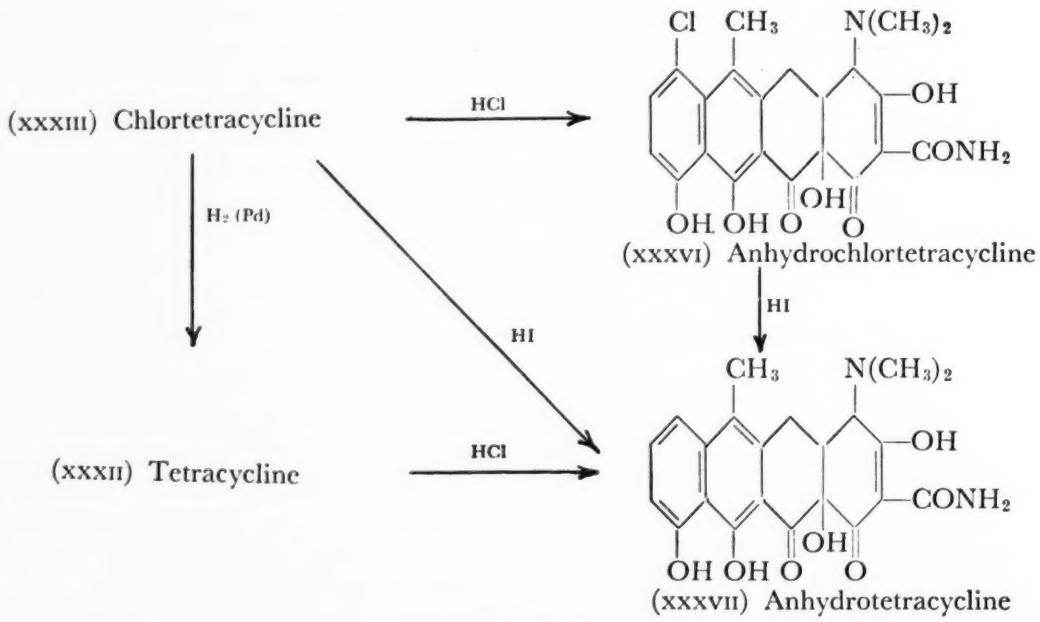
Each of these antibiotics is elaborated by certain species of Streptomyces; in addition, tetracycline is produced on a commercial scale by the hydrogenolysis of chlortetracycline. They are important and highly effective broad spectrum antibiotics which are characterized by wide antimicrobial activity against gram-positive and gram-negative bacteria, rickettsiae and the psittacosis-lymphogranuloma viruses.

Oxytetracycline, chlortetracycline and tetracycline are drugs of low toxicity and may be

administered orally, intravenously and intramuscularly. A study comparing the relative toxicities of these three antibiotics showed no pathologic changes in dogs fed either oxytetracyline or tetracycline.[303] Each drug is readily absorbed from the gastrointestinal tract and subsequently appears in the various body fluids. A large part of the drug not absorbed is recovered in the stool. The antibiotics appear in the urine in fairly high concentration during the first two hours after administration and maintain maximum concentrations for six to twelve hours.

per ml. at 25°C. Chlortetracycline hydrochloride decomposes above 210°C.; $[\alpha]_D^{25} - 240.5°$ (in water); solubility in water, 14 mg. per ml. at 25°C.; pK'a 3.4, 7.4 and 9.2.[105] Acid solutions of the antibiotic at pH 2.5 have a half-life of fourteen days at about 25°C. At pH 8.5 the antibiotic has a half-life of four hours. In the dry state chlortetracycline shows no loss in potency for long periods.[113]

In hydrochloric acid chlortetracycline (XXXIII) undergoes dehydration to form anhydrochlortetracycline (XXXVI)[105,114,115] (Scheme 5).



Scheme 5

About 10 to 20 per cent of the drug is recovered in the urine during the first twelve hours; the amount excreted during the second twelve-hour period varies considerably.[112]

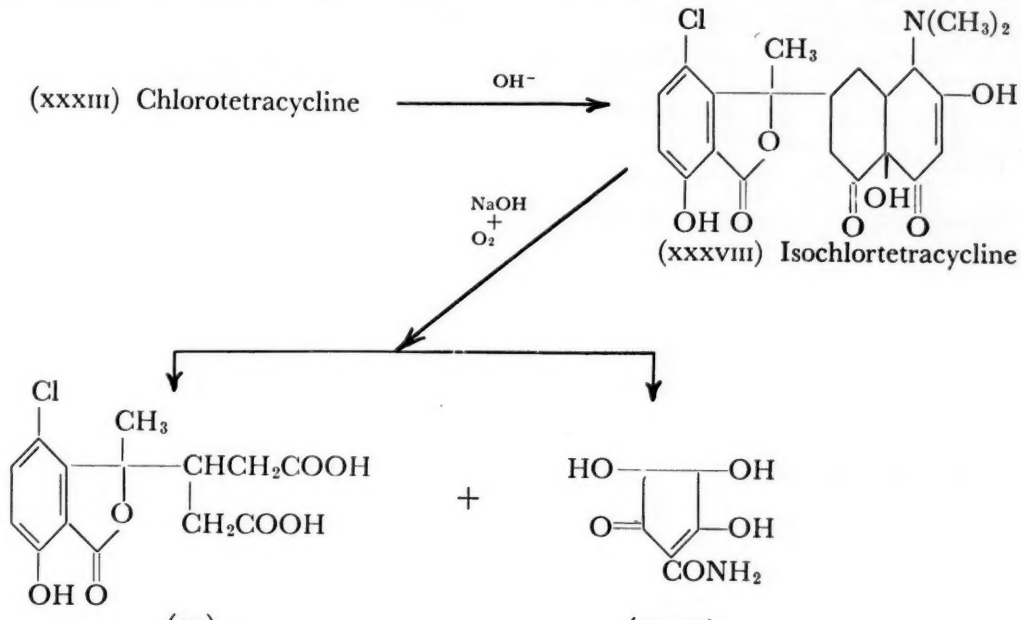
CHLORTETRACYCLINE

Chlortetracycline (Aureomycin) (XXXIII), 7-chloro-4-dimethylamino- 1,4,4a,5,5a,6, 11,12a-octahydro-3,6,10,12,12a- pentahydroxy- 6-methyl-1,11-dioxo-2-naphthacenecarboxamide, was the first of the tetracyclines to be discovered. The antibiotic was isolated from culture broths of Streptomyces aureofaciens by Duggar[108] and designated Aureomycin because of the golden yellow colors of the pigment associated with the substrate mycelium and of the antibiotic itself. Chlortetracycline is an amphoteric crystalline substance: m.p. 168°–169°C.; $[\alpha]_D^{25} - 274.9°$ (in methyl alcohol); solubility in water 0.55 mg.

The substance (XXXVI) can be converted to anhydrotetracycline (XXXVII) by removal of the chlorine atom with hydriodic acid. The same anhydrotetracycline has also been obtained by dehydrating tetracycline (XXXII) with hydrochloric acid, thus confirming certain of the structural features of (XXXVII).

Chlortetracycline is readily inactivated in dilute alkaline solution by forming isochlortetracycline (XXXVIII) through an interesting reaction (Scheme 6). The enolizable ketone at C-11 of (XXXIII) is cleaved under these conditions to give a carboxyl (acid) group which immediately lactonizes to give the phthalide, thus forming a new ring C in isochlortetracycline.[114] Prolonged action of alkali and oxygen on (XXXVIII) will ultimately cleave the dimethylamino group attached to ring A with subsequent contraction of ring A to a five-membered ring (XXXIX).[114,116]

Simultaneously, ring B is split to develop the glutaric acid side chain attached to the phthalide of (XL) which originally was part of the isochlorotetracycline molecule.[114,117,118] It is of interest that anhydrochlortetracycline possesses a fraction of the antibacterial activity of chlortetracycline.[119] This derivative (XXXVI) has been found also to be strongly inhibitory to the growth of S. aureofaciens.[119]



Scheme 6

At high concentrations chlortetracycline acts as a bactericidal drug but in the usual dilutions it behaves essentially as a bacteriostatic agent.[113] Extensive bacteriologic studies have demonstrated its effect against gram-positive and gram-negative bacteria,[120,123] against pathogenic rickettsiae,[124,125] large viruses of the psittacosis and lymphogranuloma venerum group,[126] certain fungi (Actinomyces)[127] and certain protozoa (Endamoeba histolytica).[128] Practically all strains of Proteus vulgaris and Pseudomonas aeruginosa are resistant.[123]

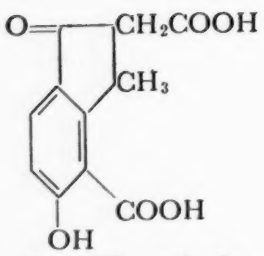
Chlortetracycline has a relatively low toxicity.[129,130] The approximate LD_{50} for intravenous administration in mice is between 50 and 100 mg. per kg.[131] Daily oral administration in patients with a variety of bacterial infections may produce occasional untoward side effects:[132] nausea, vomiting, epigastric distress, heartburn, diarrhea and pruritus ani,[133] which arise after several days of oral therapy.

OXYTETRACYCLINE

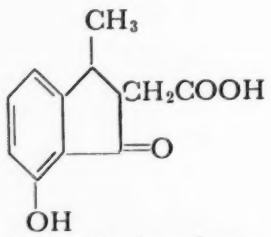
Oxytetracycline (Terramycin) (XXXIV), 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, was the second of the tetracyclines to be discovered[109] but the first to have its structure determined.[110] Oxytetracycline is produced by a species of actinomycetes which was named Streptomyces rimosus because of the cracked appearance of its growth on the surface of an agar medium.[109]

The amphoteric antibiotic crystallizes as a dihydrate, m.p. 181–182°C.; $[\alpha]_D^{25}$ + 26.5° (in methyl alcohol), solubility in water about 0.5 mg. per ml. at 25°C. The hydrochloride is a yellow crystalline substance with a bitter taste; m.p. 190–194°C.; $[\alpha]_D^{25}$ − 196.6° (in 0.1 N hydrochloric acid); pK′a 3.5, 7.6 and 9.2. It is very soluble in water; however, solutions of the hydrochloride are not sufficiently acidic (pH 2.5) to keep the amphoteric base from precipitating.[134] Oxytetracycline forms crystalline yellow-colored disodium and dipotassium salts. The sodium salt crystallizes from methyl alcohol as a dihydrate.[135] The antibiotic also forms mixed salts, very insoluble in water, with a number of pairs of bivalent metal ions such as barium-calcium and barium-magnesium. Oxytetracycline is quite stable at 37°C. Acid solutions at pH 2.5 have a half-life of twelve days; alkaline

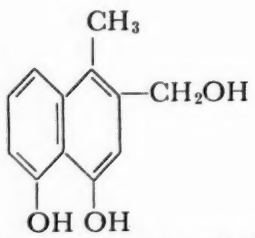
(XXXIV)
Oxytetracycline



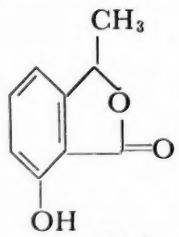
(XLI) Terracinoic acid



(XLII) Isodecarboxy-terracinoic acid

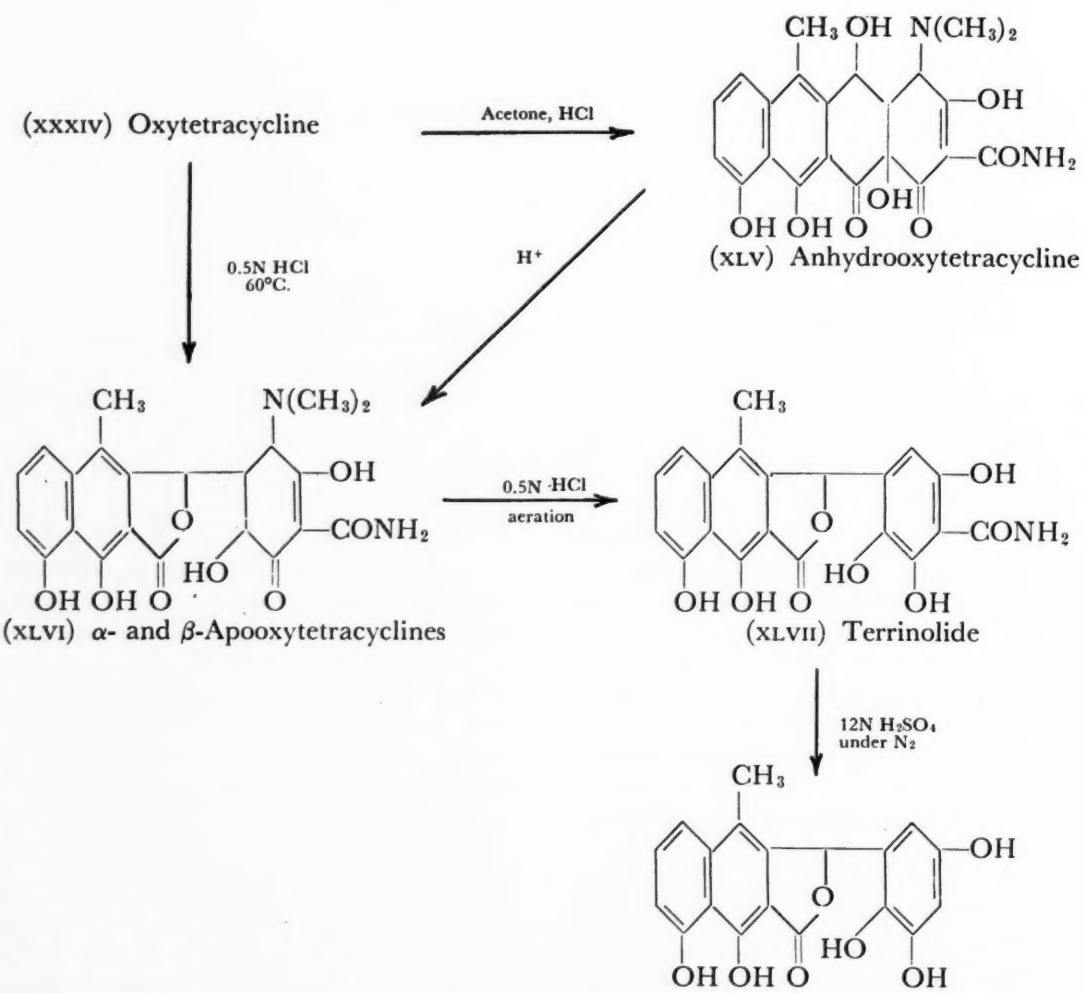


(XLIII) Terranapthol



(XLIV) 7-Hydroxy-3-methylphthalide

Scheme 7



Scheme 8

solutions at pH 8.5 have a half-life of thirty-three hours at 37°C. In dry form oxytetracycline has shown no detectable loss in biologic potency on prolonged storage at 25°C.[135]

The addition of hot aqueous alkali to oxytetracycline liberates one mole each of dimethylamine and ammonia.[136] When the antibiotic is heated in aqueous alkali in the presence of zinc, several degradation products are isolated which are the end products of alternatives modes of decomposition of the same portion of the oxytetracycline structure (Scheme 7).[137–139] None of the ring systems represented by terracinoic acid (XLI), isodecarboxyterracinoic acid (XLII), terranaphthol (XLIII) and 7-hydroxy-3-methylphthalide (XLIV) is contained in the oxytetracycline molecule.

Oxytetracycline undergoes stepwise degradation (Scheme 8) with successively stronger acids. Each product is related simply to its precursor. Since the C-12 hydroxyl hydrogen of oxytetracycline (XXXIV) is capable of enolization with the carbonyl (oxo group) at C-11 and the tertiary hydroxyl group at C-6 situated α to the aromatic ring is subject to acid-catalyzed dehydration, all the acid degradation products possess the naphthalene system and all but anhydrooxytetracycline (XLV) contain it as the 8,9-dihydroxybenzophthalide system. Anhydrooxytetracycline (XLV), like anhydrochlortetracycline (XXXVI), exhibits some biologic activity.[119,140]

Many investigators[141–145] have demonstrated the antimicrobial activity of oxytetracycline against a wide variety of aerobic and anaerobic gram-positive and gram-negative bacteria, certain rickettsiae and viruses of the psittacosis-lymphogranuloma groups.[133]

The amphoteric base and the hydrochloride of oxytetracycline show no significant differences in the body,[146–148] and both forms have been found to possess low toxicity in animal and human studies.[143,149–151] Oxytetracycline has an oral LD_{50} of 6,700 mg. per kg. in mice. The acute intravenous LD_{50} of oxytetracycline hydrochloride for mice is 178 mg. per kg. of body weight.[150] The acute LD_{50} of the hydrochloride following subcutaneous injection in mice is 600 to 650 mg. per kg.[151] In extended clinical use, gastrointestinal disturbances have occasionally been noted in some instances.[152] On prolonged therapy there have been reports of glossitis, dermatitis and pruritus ani and vulvae but these reactions are quite uncommon.[133]

TETRACYCLINE

Tetracycline (Tetracyn, Achromycin) (XXXII), 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, lacks the 7-chloro group of chlortetracycline and the 5-hydroxyl group of oxytetracycline. It is conveniently prepared by selective catalytic hydrogenolysis of chlortetracycline[105–107] (Scheme 5). Tetracycline has also been produced by fermentation of a Streptomyces species isolated from a sample of Texas soil.[153]

Tetracycline crystallizes from water-solvent mixtures as a trihydrate. The anhydrous form can be obtained by drying at 60° *in vacuo;* m.p. 170–175°C.; $[\alpha]_D^{25}$ − 239° (in methyl alcohol); solubility in water, about 0.35 mg. per ml. at 25°C.; pK′a 8.3 and 10.2. Tetracycline hydrochloride is a crystalline yellow-colored salt which melts at about 214°C.;[106,107] $[\alpha]_D^{25}$ − 258° (in 0.1 N hydrochloric acid); solubility in water 132 mg. per ml. at 26.5°C. The stability of tetracycline is comparable to that of oxytetracycline and is in contrast to chlortetracycline which is more readily affected in basic solution.[113,115]

During the preparation of tetracycline[105–107] from chlortetracycline in the presence of palladium catalyst, one mole of hydrogen is absorbed and one mole of hydrochloric acid is formed with removal of the chlorine substituent. Confirmation of the structure of tetracycline has been obtained by dehydration of the antibiotic either by methanolic hydrogen chloride[107,115] or with hydrogen iodide to yield anhydrotetracycline(XXXVII), the properties of which are closely related to those of the corresponding anhydro-compounds of oxytetracycline (XLV) and chlortetracycline (XXXVI). The substance (XXXVII) is identical with the reaction product obtained by treatment of chlortetracycline with hydriodic acid.[114,115]

Although tetracycline has not been thoroughly evaluated, it is known to possess antibiotic activity comparable to oxytetracycline and chlortetracycline.[154–156] A concentration of less than 1γ per ml. was found to inhibit the growth of Salmonella typhosa, S. paratyphi, Brucella bronchiseptica, Mycobacterium ranae, Streptococcus faecalis,[177] Klebsiella pneumoniae, S. mitis, Pasteurella multocida and certain strains of Staphylococcus aureus (Micrococcus pyogenes var. aureus).[157] It is effective against the

lymphogranuloma group of viruses.[158] Further work by other investigators[112,154,159,160] has stressed the activity of tetracycline as a broad spectrum antibiotic. The antibiotic showed little if any activity against a strain of Proteus and only limited activity against a strain of Pseudomonas aeroginosa[154] and no effect on Candida albicans.[153,154] Tetracycline, like chlortetracycline and oxytetracycline, is primarily bacteriostatic in effect; however, in high concentrations its action appears to be bactericidal.[154]

Tetracycline was found to have a low order of toxicity comparable to that of oxytetracycline and chlortetracycline when its pharmacologic properties were studied in mice, rats, rabbits and dogs.[153,154,159] The oral LD_{50} was found to be greater than 3,000 mg. per kg. in mice and rats. Moreover, single daily oral doses of 100 mg. per kg. to mice, five days per week for six weeks, produced no significant change in any of the formed elements of the blood. The animals grew at an accelerated rate. The intraperitoneal LD_{50} was 330 mg. per kg. in mice and 320 mg. per kg. in rats. The intravenous LD_{50} in mice was 170 mg. per kg., and in rats 220 mg. per kg.[159]

Patients treated with tetracycline show clinical responses sometimes accompanied by mild gastrointestinal side effects,[161] transient nausea and vomiting and slight looseness of stool. Some individuals complain of a metallic taste.[162]

ERYTHROMYCIN AND CARBOMYCIN

Two antibiotics with similar antibacterial and chemical properties were discovered almost simultaneously by three independent groups of investigators. Erythromycin was extracted from culture broths of Streptomyces erythreus, a microorganism isolated from a sample of Philippine soil,[163] and carbomycin was obtained from clarified broths of Streptomyces halstedii.[164,165] Both antibiotics are active principally against gram-positive bacteria but are also effective against a few gram-negative bacteria such as Neisseria gonorrheae and Hemophilus influenzae.[166,167] The antibiotics are active, too, against certain larger viruses[163,168,169] and some penicillin-resistant strains of Staphylococcus pyogenes var. aureus.[170] In addition, certain pathogenic protozoa have been found to be sensitive both to carbomycin and erythromycin.[171–173]

The strains of streptomyces which produce erythromycin and carbomycin both synthesize, in minor amounts, a 'B' component of the same antibiotic. The antibiotic spectrum of carbomycin B resembles closely that of carbomycin itself.[174] Though the intravenous toxicity of the B component is somewhat lower than that of carbomycin, the acute toxicities of the two antibiotics by intraperitoneal, intramuscular, subcutaneous and oral route are quite comparable. The microbiologic spectrum of erythromycin B is also similar to that of erythromycin but is only about 75 to 85 per cent as active.[175]

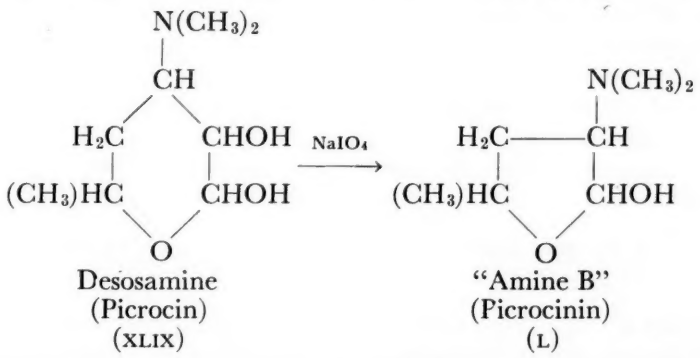
The toxicites of carbomycin and erythromycin are quite low. No eighth cranial nerve damage in cats has been detected with either antibiotic. Carbomycin hydrochloride has an intravenous LD_{50} in mice of 500 to 600 mg. per kg. of body weight; erythromycin has about 400 to 500 mg. per kg. by the same route. Intravenous injections in dogs at concentrations of 200 mg. of carbomycin per kg. for five days were well tolerated by these animals.[176] Dogs and cats tolerated 50 mg. of erythromycin per kg. for two to three months;[177] however, a delayed toxicity has been noted with erythromycin when young guinea pigs were used as test animals.[178]

Both antibiotics are white crystalline substances which are well characterized but their chemical structures are complex and not completely elucidated.

Erythromycin (Ilotycin, Erythrocin) is a weak basic crystalline substance, pK′a 8.6; m.p. 135–140°C.; $[\alpha]_D^{25} - 73.5°$ (in methanol). It readily forms salts with acids of which the hydrochloride, stearate and glucoheptonate are well characterized. It forms, in addition, an interesting ethyl carbonate derivative which is biologically active. The antibiotic is soluble in organic solvents, moderately soluble in ether and only slightly soluble in water. It is stable in neutral solutions but below pH 5 the drug loses its activity rather rapidly.[179]

Mild acid hydrolysis of erythromycin yields dimethylamine, propionaldehyde, propionic acid, a high melting (207–208°C.) solid called erythralosamine, $C_{29}H_{49}NO_8$, and a high boiling liquid named cladinose, $C_8H_{16}O_4$.[179,180] Further hydrolysis of erythralosamine or strong acid hydrolysis of erythromycin produces a mixture of degradation products from which desosamine (XLIX), a crystalline hydrochloride of a basic substance with the composition $C_8H_{17}NO_3 \cdot HCl$,[179,181] can be isolated. Surprisingly, the identical substance (XLIX), also called picrocin, has been recovered from the degradation reac-

tion of picromycin,[182] an antibiotic with an antibacterial spectrum resembling erythromycin, which was isolated from a strain of Streptomyces fellens.[183] The substance (XLIX) has been shown to be a novel amino sugar, 3-dimethylamino-4-deoxy-5-methylpentopyranoside. Oxidation of (XLIX) with sodium periodate produces a new amino sugar, (L), with one less carbon atom. This substance has been named "amine B,"[181] as well as picrocinin.[182] The stereochemical configuration of neither amino sugar (XLIX) nor (L) has been determined. The structures of cladinose and erythralosamine also are incompletely known; thus only a partial structure of erythromycin (LI) can be postulated from these unfinished studies.[179]

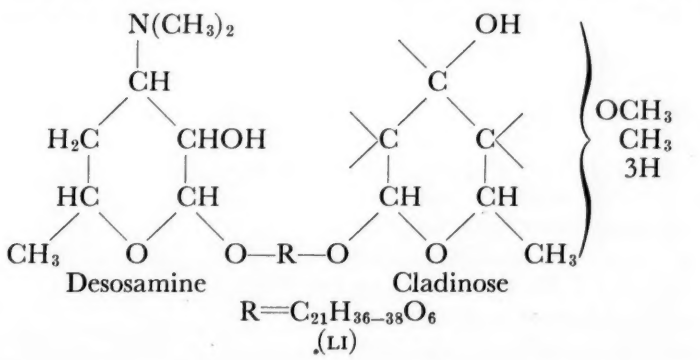


Desosamine (Picrocin) (XLIX) — "Amine B" (Picrocinin) (L)

Erythromycin B is similar to erythromycin in practically all its physical properties. The B component is a comparable basic substance pK′a 8.5; $[\alpha]_D^{25} - 78°$ (in ethyl alcohol) and melts at 191–195°C. The infrared absorption spectra of the antibiotics show only minor differences.[175]

Carbomycin (Magnamycin)[184,185] is a crystalline monobasic substance, pK′a 6.8, with the approximate molecular formula $C_{41-42}H_{67-69}NO_{16}$ so far as known.[184] It crystallizes well from methanol; m.p. 212–214°C., $[\alpha]_D^{25} - 58.6°$ (in chloroform). The antibiotic is readily soluble in most polar organic solvents but is virtually insoluble in hydrocarbons and water. It forms stable salts with mineral acids; the hydrochloride is readily soluble in water in contrast to the periodate salt which is quite insoluble. Several biologically active derivatives, for example the diacetyl, oxime and thiosemicarbazone, have been prepared. Hydrogenation of carbomycin over palladium catalyst results in prompt absorption of hydrogen and yields crystalline tetrahydrocarbomycin; continued hydrogenation, however, produces a wholly inactive compound.

Crystalline carbomycin in the absence of moisture shows no evidence of decomposition after storing for long periods in the dark at room temperature. Aqueous solutions of the antibiotic are stable between pH 5–7 and show no measurable loss in microbiologic activity after eleven days at 25°C. More acidic solutions, namely pH 3, and more basic solutions, pH 9, are half inactivated in the same period.



Desosamine — Cladinose

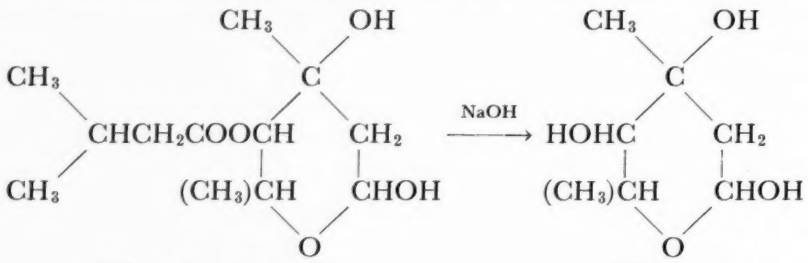
R=$C_{21}H_{36-38}O_6$

(LI)

Alkaline hydrolysis of carbomycin yields one mole each of acetic and isovaleric acids and dimethylamine per mole of antibiotic. Hydrolysis in dilute acid forms a crystalline base of the formula $C_{29-30}H_{47-49}NO_{12}$ and an oily neutral substance $C_{12}H_{22}O_5$.[184] This viscous colorless oil is the 4-isovaleryl ester of a new unusual branched chain deoxysugar[186] which has been named mycarose. 4-Isovalerylmycarose has the structure (LII); however, the formula should

be regarded as devoid of configurational implications.

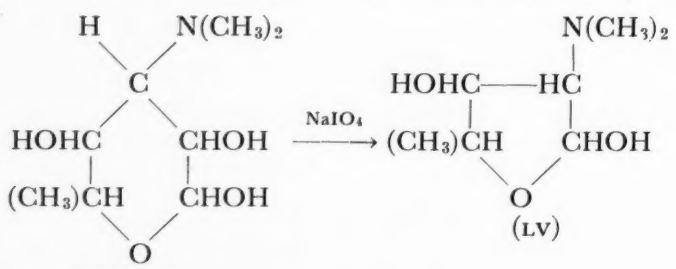
More vigorous acid hydrolysis of either carbomycin or of the basic fragment $C_{29-30}H_{47-49}NO_{12}$ yields the unique amino sugar, mycaminose (LIV) with the empirical formula $C_8H_{17}NO_4$.



4-Isovalerylmycarose (LII) — Mycarose (LIII)

HCl. The substance has been shown to be the hydrochloride of a 3-dimethylamino-3-deoxy-5-methylpentopyranoside[187] which is closely related to the dimethylamino sugar isolated from erythromycin and picromycin. Oxidation with periodate salt yields a mole of formic acid per mole of amino sugar and a new dimethylamino sugar, $C_7H_{15}NO_3$ (LV), with one carbon atom less than mycaminose.[187]

Carbomycin B has the probable molecular formula $C_{41-42}H_{67-69}NO_{15-16}$.[174] It shows a marked similarity to carbomycin in most of its



Mycaminose (LIV) — (LV)

chemical and biological characteristics. Carbomycin B can be distinguished from carbomycin by its absorption spectrum which gives evidence of an α-β-γ-δ-unsaturated carbonyl system, in contrast to the α-β-unsaturated carbonyl system exhibited by carbomycin. The B component is much more soluble in organic solvents. It forms many of the derivatives of carbomycin itself and on hydrolysis also yields isovalerylmycarose (LII) and mycaminose (LIV).

FUMAGILLIN

Fumagillin (H-3) is a potent amebicide[188] but not much importance was attached to earlier announcements of its discovery by two independent groups[189,190] who found it capable of inhibiting bacterial viruses. The substance was isolated from cultures of Aspergillus fumigatus and designated H-3 by one group[188] and phagopedin sigma by a second group of investigators.[191]

Fumagillin has little antibacterial and antifungal activity and no antiviral activity when tested in mice with MM virus and influenza PR8A infections.[190] The antibiotic produces inhibitory effects *in vitro* against a strain of Endamoeba histolytica (NIH200) at dilutions as high as 1:131,000,000 in mixed bacterial flora. In contrast to other amebicides, it would appear that fumagillin exerts its action directly on E. histolytica.[189,192]

Young rabbits were cleared of experimental infections of E. histolytica with a total oral dose of about 100 mg. of fumagillin per kg. of body weight, divided over a two-day period.[188] The antibiotic possesses considerable amebicidal activity also in monkeys[1 3] and in humans.[173,192] The LD_{50} by the subcutaneous route in mice is approximately 800 mg. per kg. and upward of 3,000 mg. per kg. was tolerated orally.

Fumagillin is a light yellow crystalline substance, m.p. 189–194°C.; $[\alpha]_D^{25} - 26.6°$ (in methyl alcohol). The antibiotic is a dibasic acid which forms a methyl ester and a monopotassium salt.[194] Fumagillin is soluble in most organic solvents and in dilute alkaline solutions; it is insoluble in saturated hydrocarbons, water and dilute

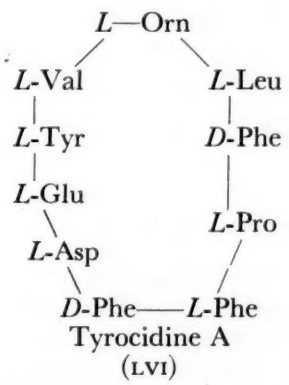
acids. The approximate empirical formula, $C_{26-27}H_{34-36}O_7$, has been suggested for the antibiotic.[194,195] Under mild alkaline conditions, the antibiotic yields a highly unsaturated acid, namely, 2, 4, 6, 8-decatetraenedioic acid: HOOC·CH=CH·CH=CH·CH=CH CH=CH·COOH, probably the first time this acid has been isolated from a natural source. Chemical evidence suggests that fumagillin is the mono-ester of decatetraenedioic acid[195,196] and an alcohol $C_{16}H_{26}O_4$.[197] This alcohol is isolated from fumagillin in its hydrated form after successive acid and alkaline hydrolyses of the antibiotic. The alcohol contains a methoxyl and hydroxyl group but no carbonyl function.[197] With the partial structure so far advanced, elucidation of the complete structural picture is fairly well assured.

THE POLYPEPTIDE ANTIBIOTICS

Tyrothricin and Gramicidin

Tyrothricin, a mixture of polypeptide-like substances possessing antibacterial activity, was isolated from strains of Bacillus brevis[198] by Dubos in 1939 during one of the earliest planned investigations to find an organism which would destroy pathogenic bacteria. Subsequent fractionation showed that tyrothricin could be separated into the neutral gramicidins and basic tyrocidines.[199] These groups of substances exhibit different antibacterial properties. Both groups are active against gram-positive microorganisms, in addition tyrocidine exhibits slight activity against gram-negative bacteria. Both groups are toxic to experimental animals when given parenterally but appear to have lower toxicity when given orally.[200] They are injurious to the blood cells and both hemolyze erythrocytes.[201] A somewhat less toxic derivative of gramicidin can be formed by the addition of formalin to the antibiotic. The resulting methylol gramicidin shows about one-sixth the hemolytic power of gramicidin when tested *in vitro*, and about one-tenth the toxicity to mice on intravenous injection.[202,203]

A vast amount of work has gone into purification of these groups of antibiotics.[204] More recent studies using countercurrent distribution have shown that crystalline tyrocidine hydrochloride is composed of a family of polypeptides with three major components,[205] designated tyrocidines A, B, and C. The three peptides differ with respect to their tryptophan or tyrosine content. Tyrocidine C contains a large amount of tryptophan whereas tyrocidine A contains none. Tyrocidine A has a molecular weight[206] of about 1270 and the empirical formula $C_{66}H_{87}N_{13}O_{13}$·HCl.[205] But two functional groups are present in tyrocidine A, namely, the hydroxyl group of a single tyrosine residue and the δ-amino group of the ornithine moiety which accounts for the basicity of the molecule. It is a cyclic peptide[205] with no free carboxyl group or α-amino groups. Hydrolyzates of the antibiotic have shown the presence of valine, tyrosine, leucine, proline, ornithine, glutamine, asparagine and three phenylalanine molecules. Tyrocidine A was subjected to a series of partial hydrolyses and a sufficient quantity of polypeptides was isolated, by countercurrent distribution and ion-exchange chromatography,[207] to permit formulation of an unambiguous sequence for the amino acids in the molecule (LVI).[207*]



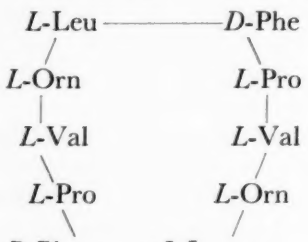
Tyrocidine A
(LVI)

Along with the tyrocidines, the tyrothricin mixture contains at least four other closely related substances, three of which have been crystallized and designated gramicidins A, B and C. The gramicidins are neutral substances possessing neither free amino nor carboxyl groups[208] and are characterized by the presence of ethanolamine (2-aminoethanol-1).[209,210] Besides ethanolamine, gramicidin A shows the presence of *D*-leucine, *L*-tryptophan, *DL*-valine, *L*-alanine and glycine.[208,211,212] Gramicidin B contains these amino acids and phenylalanine in addition, while gramicidin C contains tyrosine instead of phenylalanine.[213]

Gramicidin S ('Soviet gramicidin') is a polypeptide antibiotic elaborated by a different strain of B. brevis.[214,215] However, the antibiotic closely resembles tyrocidine rather than grami-

* In general the first three letters are used to designate the amino-acid residue.

cidin in its chemical and antibacterial properties. The molecule contains the non-natural amino acid *D*-phenylalanine, *L*-ornithine (which is seldom encountered in a naturally occurring peptide structure), *L*-valine, *L*-leucine and *L*-proline.[216] The unit possesses two free amino groups contributed by the δ-amino group of the ornithine residue[217–219] and no free carboxyl groups.[219] In all probability the antibacterial substance is a cyclic decapeptide, or a twice repeated five amino acid sequence, in the form of two pentapeptides joined in a ring[216] (LVII).



Gramicidin S
(LVII)

Although it has been stated that the biologic activity of gramicidin S is dependent upon its cyclic structure,[220] this view has been challenged recently.[221]

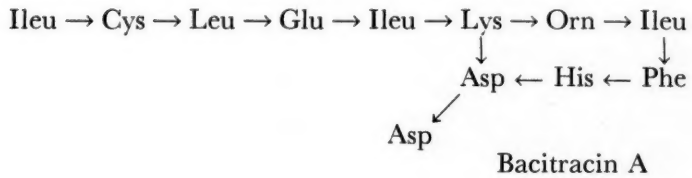
Bacitracin

Bacitracin was isolated in 1945 from cultures of Bacillus licheniformis,[222] a microorganism of the B. subtilis group. The name "bacitracin" was suggested when a particularly potent strain of the microorganism was isolated from a patient named Tracy. The antibiotic was also isolated from a strain of B. subtilis[223] independently, by a group of investigators who referred to the antibiotic as "ayfivin."[224] This name was withdrawn when it was noted that several constituents present in "ayfivin" were identical with those in bacitracin.[225–227]

Bacitracin has marked antimicrobial action on gram-positive bacteria[222] and shows some synergistic action with the other antibiotics, particularly penicillin.[228,229] Production of the antibiotic by surface cultures was later supplanted by growth of submerged cultures of B. subtilis in synthetic media.[230] With intensive purifications, samples of bacitracin have given a potency of 60 units per mg. but material of this purity rapidly declines in potency to a level of 40 to 45 units per mg. Because of these objections, attempts have been made to prepare more stable forms of the antibiotic, such as zinc bacitracin[231] and bacitracin methylene disalicylate.[232] Bacitracin has merit as a topical medicament and has proved useful in certain surgical infections.[229] In contrast to penicillin, bacitracin rarely sensitizes patients.[232] Like most polypeptide antibiotics, however, bacitracin is nephrotoxic and patients who are treated parenterally with the drug must be checked for evidences of kidney damage such as albumin in the urine and nitrogen retention in the blood.[133]

Bacitracin is a labile compound and readily affected by alkali, strong acid and by formaldehyde. However, studies by such mild methods of separation as countercurrent distribution, paper chromatography and, to some extent, paper electrophoresis have provided considerable knowledge of the complex composition of bacitracin. Commercial bacitracin appears to be a complex peptide comprised of at least five components designated bacitracin A, B, D, E and F.[233] In addition to these components A′, C, G, F_1, F_2 and F_3 have been detected in samples of bacitracin originally called "ayfivin."[234]

The main peak exhibited in countercurrent distribution studies, and thus the chief component of bacitracin mixtures, has been assigned bacitracin A. In its purified form it has been found to have a molecular weight of about 1,470 which suggests an empirical formula best represented[235,237] by $C_{65}H_{103}N_{16}O_{17}S$. Component A is quite sensitive to acids and alkalis. At about pH 8 it is slowly transformed into bacitracin F.[235] Acid hydrolysis of the main component A yields a mixture which on a chromatographic map indicates the presence of *L*-leucine, *L*-isoleucine, *L*-cystine, *L*-histidine, *L*-lysine, *D*-phenylalanine, *D*-ornithine, *D*-glutamic acid and *DL*-aspartic acid.[236,237] Considerable thought and painstaking work has been directed toward experiments which would yield information regarding the sequence of these amino acids in the bacitracin A molecule.[238–241] Particularly significant has been the experimental evidence leading to sound conclusions regarding the arrangement of amino acids isolated from partial hydrolyses of bacitracin A. Numerous peptide fragments and their dinitrophenyl derivatives were isolated and characterized by a combination of countercurrent distribution, paper chromatography, zone electrophoresis and ultimate analysis.[242] The partial hydrolysis products were hydrolyzed further with acid to yield their component amino acids. These acids were subsequently

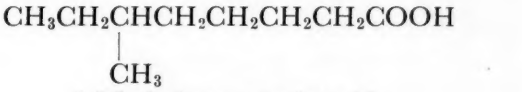


Bacitracin A
(LVIII)

identified by countercurrent distribution and paper chromatographic methods.[238,239] The interpretation of these results has led to the scheme indicated in (LVIII) where → signifies a C–N bond.[238,240,243,244] The unique cross linking of the lysine residue where it is joined at three different positions was interpretated through a study of the partial hydrolysis of the dinitrophenyl derivative of bacitracin A.[245]

Polymyxin

The discovery of polymyxin was made almost simultaneously by three independent groups of investigators[246,247,248] who isolated the antibiotic from several strains of Bacillus polymyxa. The multiple nature of this group of closely related antibacterial substances was soon recognized[249] and on the basis of amino acid composition, has been shown to be an optically active isopelargonic (nine-carbon) acid, namely, (+)-6-methyloctan-1-oic acid (LIX):[258,259]



6-Methyloctan-1-oic acid.
(LIX)

However, while the amino acid spectrum of polymyxin B_1 and B_2 is the same,[254] subsequent investigations have disclosed that the two polypeptides differ in the nature of the fatty acid component present in each. Polymyxin B_1 contains the methyloctanoic acid (LIX) but the B_2 component contains an isooctanoic acid, $C_8H_{16}O_2$, whose exact structure has not yet been determined.[255] A careful examination of the

TABLE II
COMPONENTS OF THE VARIOUS POLYMYXINS

Polymyxin	(+)–6–Methyloctanoic Acid	*D*-Leucine	*L*-Threonine	*D*-Serine	*D*-Phenylalanine	*L*-α, γ-Diaminobutyric Acid
A	+	+	+	−	−	+
B	+	+	+	−	+	+
C	+	−	+	−	+	+
D	+	+	+	+	−	+
E	+	+	+	−	−	+
Circulin	+	+	+	−	−	+

shown in Table II,[250] the various polymyxins are designated A, B, C, E,[251] and D.[247] They all contain *L*-α,γ-diaminobutyric acid, *L*-threonine[252] and the same optically active $C_9H_{18}O_2$ fatty acid.[252,253] While no evidence has emerged that any one strain produces more than one class of antibiotics it has been found that the strain which elaborates polymyxin B actually produces two slightly different polymyxin peptides[254] called B_1 and B_2.[255] Circulin,[256] an antibiotic produced by cultures of B. circulans, is also closely related to the polymyxins. It has the qualitative composition[257] of polymyxins A and E, as indicated in Table II.

The fatty acid present in all the polymyxins acid hydrolyzates of polymyxin B_1 has revealed that the molecule is composed of 6 moles of *L*-α, γ-diaminobutyric acid, two of *L*-threonine, one of *D*-phenylalanine, one of *L*-leucine and one of 6-methyloctanoic acid.[255]

The molecular weight of polymyxin B_1 was found to be 1150 ± 50. This value is in good agreement with the proposed empirical formula $C_{56}H_{99}N_{16}O_{14}$ which is based upon evidence for a free carboxyl group and the assumption that all the components in the molecule are linked together to form a cyclic polypeptide.[252,255]

The various polymyxins display a selective but similar spectrum of activity against gram-negative bacteria,[133,260,261] although there are

slight differences in antibacterial activity.[141,142] Polymyxin B is particularly useful in gram-negative bacillary infections and in infections due to Pseudomonas aeruginosa and Hemophilus influenzae which fail to respond to other antibiotics. Moreover, resistance of microorganisms to polymyxin develops very slowly. These favorable characteristics, however, are offset by the inherent nephrotoxicity of polymyxin,[262] which is, however, less pronounced in B and E[263] than in A and D. As a consequence of this, and because of certain production advantages, polymyxin B is used almost exclusively. Polymyxin B causes occasional nephrotoxicity and neurotoxicity and for this reason parenteral administration requires certain precautions. The antibiotic is free of toxic effects following oral or topical administration. Polymyxin is available also in combinations with oxytetracycline, bacitracin, sulfonamides, etc.[133]

Neomycin

The elaboration product of Streptomyces fradiae[264] has been shown to be a heterogeneous mixture[265] of at least three antibiotics, designated the "neomycin complex." The antibiotic has a biologic activity comparable to streptomycin.[266,267] The microorganism S. fradiae also forms an antibiotic called fradicin, $C_{30}H_{34}N_4O_4$,[268] which has antifungal properties but no activity upon bacteria.[269] A previously undescribed strain of S. albogriseolus has been found to produce the "neomycin complex" free of an antifungal component.[270]

Neomycin is active against many gram-positive, gram-negative and acid-fast bacteria.[264] Its antibacterial spectrum resembles that of streptomycin but streptomycin-sensitive and streptomycin-resistant strains of bacteria, including those of Mycobacterium tuberculosis var. hominis, are inhibited by neomycin.[271] Bacteria appear to develop resistance to neomycin at a slower rate than with streptomycin.[272,273]

In the standardization of neomycin by the Food and Drug Administration[274] the cup method is used with Staphylococcus aureus 9144 ATCC as the test organism and a neomycin sulfate standard assigned 1000γ per mg.: equivalent to 333 units per mg. of pure base. Neomycin, commercially prepared, has a potency of about 250 units per mg. Certain preparations have been found to contain largely neomycin B,[275] with smaller quantities of neomycins A and C; other commercial preparations have been reported to be a single entity.[276]

Preparations containing about 200 units per mg. showed an LD_{50} of about 50 mg. per kg. of body weight when injected intravenously in mice.[277] Acute toxicity tests of a single component material[276] indicate an LD_{50} of approximately 80 mg. per kg. intravenously, and 400 mg. per kg. subcutaneously in mice. Thus on a weight basis neomycin is more toxic than streptomycin. Preparations of neomycin exert a beneficial effect on tuberculosis in guinea pigs.[278] The antibiotic, however, produces kidney damage and loss of weight in guinea pigs during prolonged administration.[278,279] Pure neomycin B and C show approximately the same acute toxicity and protective effect in mice infected with tuberculosis.[280]

TABLE III
COMPARISONS OF PROPERTIES OF NEOMYCIN

	Neomycin A HCl	Neomycin B HCl	Neomycin C HCl
Biopotency by Klebsiella pneumoniae	20 U/mg.	265 U/mg.	180 U/mg.
Biopotency by Bacillus subtilis	1700 U/mg. 710 U/mg.	86 U/mg.	121 U/mg.
$[\alpha]_D^{25}$ (in water)	+83°	+54°	+80°

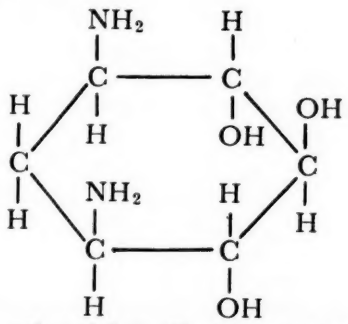
The antibiotic has been used in certain gram-negative urinary tract infections due to Aerobacter aerogenes, Proteus, some strains of Pseudomonas aerugenosa, and in the treatment of pyogenic skin infections. Kidney damage[281] and progressive deafness[281,282] have been observed in patients with tuberculosis during treatment with neomycin. The antibiotic is more frequently used in combination with other substances, e.g., cortisone, hydrocortisone, oxytetracycline, gramicidin, bacitracin, etc.

Investigations into the chemistry of neomycin have been accompanied by considerable difficulty because of problems in the isolation and purification of the individual components.[283] In addition, the potencies of these components differ somewhat depending upon the method and microorganisms used to assay them. Some distinguishing features of the three neomycin components A,[284] B[275,285] and C[285] are given in Table III.[285]

Neomycin is a basic antibiotic which readily forms salts with a variety of acids. The molecule contains nitrogen in the form of primary amino groups. Highly purified neomycin, which appears to contain only one component;[276] is

extremely stable to alkali. At pH 2.0, it is stable for twenty-four hours at room temperature.

When neomycin B and C were subjected to degradation studies by heating in moderately strong mineral acids, an unexpected biologically active substance was obtained which is closely comparable to neomycin. The substance was called neamine[286] but subsequent work[287,288] showed it to be identical with neomycin A,[284] which indeed may occur as a minor component of the neomycin complex.[289] The molecular formula of the substance is a multiple of its empirical composition, $C_6H_{12-14}N_2O_3$.[286] Further hydrolysis of neamine (neomycin A) in strong acids at elevated temperatures has yielded a fragment identified as the dihydrochloride of a $C_6H_{14}N_2O_3$ substance, the *meso* isomer of 1,3-diamino-4,5,6-trihydroxycyclohexane (LX).[290]



1,3-Diamino-4,5,6-trihydroxycyclohexane
(LX)

Hydrolysis of neomycin B and C in methanol containing hydrogen chloride produces two fragments.[285] One of these fragments, which is isolated from both neomycin B and C, is neamine.[287] While certain investigators[286] have suggested the formula $C_6H_{12-14}N_2O_3$ for neamine, this substance must contain more than six carbon atoms to account for the presence of (LX) and a second moiety in the molecule.[287]

It is of interest that a diaminopolyhydroxycyclohexane moiety is also present in streptomycin in the form of streptidine (XIV). Moreover, many of the similar biochemical and pharmacological properties of neomycin and streptomycin may be due to still other chemical analogies in both molecules. It has been suggested that neomycin also contains a diaminodeoxy sugar and a pentose[285] which might parallel the N-methyl-glucosamine (XVI) and streptose (XV) fragments in streptomycin. However, it will require considerably more chemical studies to clarify the structure of neomycin.

VIOMYCIN

Viomycin (Viocin, Vinactin) is a tuberculostatic antibiotic which is produced by a group of closely related strains of Streptomyces vinaceus (Actinomyces vinaceus),[291] S. puniceus[292] and S. floridae.[293,294] During the fermentation, S. vinaceus produces two and possibly three closely related antibiotics designated Vinactin A, B and C, which have not been well characterized.[295] Viomycin is an antimicrobial agent effective particularly against both streptomycin-sensitive and streptomycin-resistant strains of tubercle bacilli. It is currently in use as an adjunct in the treatment of tuberculosis.

Viomycin is a drug of moderately low toxicity. The acute intravenous LD_0 for mice was found to be 120 mg. per kg. of body weight, while its acute intravenous LD_{50} is 165 to 200 mg. per kg. Experimental animals, rats, cats, dogs, etc., have shown little or no toxicity with dosages equivalent to 60 mg. per kg. or less of viomycin administered daily for prolonged periods of time. Disturbances of posture and gait were observed in cats receiving daily subcutaneous injections of 50 to 100 mg. per kg. over a six-week period.[296,297]

Viomycin sulfate in the dry form may be stored at room temperature for twenty-four months without appreciable loss of potency. Solutions of viomycin sulfate at pH 5.6 may be stored at room temperatures under sterile conditions for seven days without significant loss.

Viomycin is a polyacidic strong base of the empirical formula $C_{18}H_{31-33}N_9O_8$.[298] The free antibiotic base has not been prepared but several salts such as sulfate, hydrochloride[294] and picrate[298] have been crystallized. Hydrated viomycin sulfate decomposes at about 280°C.; $[\alpha]_D^{25} - 32°$ (in water). The salt is highly soluble in water and virtually insoluble in most organic solvents.[292] Qualitative tests indicate the absence of a free α-amino carboxyl group. The antibiotic possesses one primary amino group as indicated by Van Slyke determination, several amino acids bound through peptide linkages, a guanidino group, and gives a positive test for creatinine. Vinactin A and B appear to be similar peptides in which guanidino and creatinine groups have been reported to be present. Preliminary studies suggest the presence of creatinine in Vinactin C.[295]

Viomycin is relatively stable to strong acids; however, vigorous acid hydrolysis completely inactivates the antibiotic. Paper chromatograms of strong acid hydrolyzates of Vinactin A showed evidence of serine, lysine, alanine and glycine as well as glutamic and aspartic acids.[295] Products of the acid hydrolysis of viomycin[298,299] are carbon dioxide, ammonia, urea, *L*-serine, α,β-diaminopropionic acid, an unidentified guanidino component of approximate formula $C_5H_{10}N_3$ and β-lysine (β,ϵ-diamino-n-caproic acid: $NH_2{\cdot}CH_2CH_2CH_2CH(NH_2)CH_2COOH$), identical with the amino acid isolated from streptothricin,[299] streptolin[300,301] and roseothricin.[302]

CONCLUDING REMARKS

New antibiotics have invariably provided unique chemical structures which have challenged the ingenuity of the organic chemist to unravel their structural features and to prove such structures by synthesis, as was so successfully achieved with chloramphenicol. Even in this limited discussion of the chemistry of antibiotics we have noted the existence of a non-natural amino acid and amino sugar, respectively, in the molecules of penicillin and streptomycin. In addition, we have noted the unexpected presence of the nitrobenzene group in chloramphenicol; the new type of four ring-system forming the skeletons of oxytetracycline, chlortetracycline and tetracycline; the unique amino sugar moieties in both carbomycin and erythromycin; the highly unsaturated decatetraenidioic acid in fumagillin; the new amino acid, β-lysine, in viomycin; the multiplicity of components in the tyrothricin, bacitracin and polymyxin complexes, and the cyclic arrangements of the amino acids in these polypeptide antibiotics.

As we progress toward the goal of the universal antibiotic, we may expect these patterns to repeat themselves. In addition, we can anticipate new mycologic and bacteriologic technics to reveal new antibiotics, new biochemical engineering advances to produce them in pure form and in large amounts, and perhaps new medical approaches to exploit their chemical and biologic properties against microorganisms which are unaffected by presently known antibiotics.

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Mode of Action of the Antibiotics*

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An antibiotic, although originally defined as a substance produced by one microorganism which inhibits the growth of another, has come to mean a substance produced by a living organism (micro or not) which inhibits or kills another.[1] Note that this definition says nothing about the conditions under which such inhibition shall occur, and does not imply that an antibiotic necessarily has any clinical usefulness whatsoever. The situation actually is that there are many thousands of different kinds of organisms which produce substances of varied nature capable of inhibiting other organisms. Among these thousands it has been possible to isolate and to determine the chemical structure of some hundreds.[2] At least sufficient numbers are known in detail, so that it is apparent that there is no chemical structure common to all, and that essentially every major class of chemical compounds is represented. It is a necessary property of all of the antibiotic substancess that they possess a degree of specificity, that is, that they inhibit one organism and not another, or at least that they inhibit one more than another. Sometimes the specificity is broad, sometimes narrow; but it must always exist in some degree (otherwise the organism producing it would be killed before the substance was formed in detectable amount).

Among these hundreds of chemically defined antagonists there are some, perhaps about twenty, which possess a degree of specificity so great that they can be used within the human body to exert their inhibiting or lethal effect on microorganisms without comparable damage to the host. These are clinically useful. Five of such substances have been available for a sufficiently long time and have received sufficient biochemical study so that something is known, in chemical terms, of their "mode of action."

MEANING OF MODE OF ACTION

The subject of the "mode of action" of antibiotics is frequently somewhat confused because different meanings are attributed to the term.[3–6] The question is really, "How does the drug act?" At one level of information one may inquire as to whether it is bacteriostatic or bactericidal; whether it attacks the growing cell or the nongrowing cell, and various similar questions. All of this information may be called mode of action, and it does indeed tell one something about the action of the substance. This type of information is readily available for hundreds of antibiotics.

The next stage in the inquiry on "How does the drug act?" is to determine the answer to two questions: first, what is the nature of the lesion introduced into the microorganism by the presence of the antibiotic?; second, in those antibiotics which possess the unique and surprising property of being able to enter the body to kill off the microorganisms without harm to the host, we need to inquire as to what is the basis of this marked and very useful specificity? How, indeed, is this possible?

There is a further stage of inquiry regarding how the drug acts; a consideration of the precise details of the process. However, research on antibiotics has not reached this level of inquiry and, in fact, in only a few instances has it gone beyond the initial inquiries regarding bacteriostasis or specificity. The purpose of this article, however, is to consider in a reasonably organized fashion, "mode of action" in terms of the two questions mentioned: the nature of the lethal lesion in the microorganism, and the reasons why some of the substances can be used in the human body. The penicillins, the streptomycins, chloramphenicol and the tetracyclene group have been subject to widespread clinical experience under a wide variety of circumstances over a fairly long period of time and are known to be effective drugs. Some studies have been made on their mode of action and these we wish to consider. There is further an array of substances —viomycin, neomycin, candicidin, erythromycin, carbomycin and the like—some of which are of great promise but are either too recent

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or too difficult in practice so that experience with them in the community at large is still small. The modes of action of these are not known and hence will not be considered further except in one or two instances which will be mentioned later. There are several agents, such as bacitracin and gramicidin, whose toxicity to the body is too high for internal use but which do find a use in practice under restricted circumstances and are, therefore, of medical import. These will be omitted from our study.

PROPERTIES COMMON TO ALL MAJOR TYPES OF ANTIBIOTICS

Considering the "mode of action" of the four main groups of antibiotics (penicillins, streptomycins, chloramphenicol and the tetracyclenes) there are certain aspects of their action which all of these substances have in common.

They are all adsorbed to or absorbed by the susceptible cells. In the cases adequately studied there is a "specific" irreversible absorption, although this may not be true for all. The action of all is primarily biochemical, not physical, and they all seem to interfere with a different reaction or reaction type within the cell. A great many reactions are not in the least affected by them, and a wide array of cell processes continues in their presence.

These substances are all relatively complex molecules containing a fair number of substituent groups which may bear a resemblance to other structures in the cells. It seems reasonable that molecules of this complexity may interfere with reactions in which similar groups are involved, if only the case of "the butter not suiting the works." Some of these interferences may be quite specific and directly related to how the antibiotic kills the organism, but some may be due to the fact that similarity in structure causes interference in reactions in which such structure is of importance, even though this has nothing to do with its mode of action. To distinguish between the antibiotic effects and what one may call the structural effects of the antibiotic, there are two criteria in use at present. These are: (1) The effects observed must be evident for the antibiotically active forms of the antibiotic but show no reaction with structurally similar derivatives which possess no antibiotic activity. (2) The concentrations of the antibiotic required to act on a given reaction must be comparable to those required to inhibit growth. More stringent criteria of antibiotic action may be required later; for the moment these suffice.

All of the four major groups of antibiotics act by inhibition of a particular biochemical reaction within the cell, and not by a general interference with a variety of reactions. In short, it appears that their action is chemically pinpointed to a particular reaction (or a few related types) within the susceptible cell. It is, of course, axiomatic that any drug must be adsorbed by and react with the tissue it is to influence, and the antibiotics are no exception. These factors of adsorption, penetration and reaction are similar to those encountered in other drugs and, with the exception of the case of the streptomycins, seem to have no particular bearing upon their mode of action, although they do of course, affect activity of the drug in practice.

It is now necessary to define, insofar as is yet possible, the specific reactions inhibited by each of the antibiotics. Since these differ it is necessary to consider each separately.

SPECIFIC PROPERTIES OF INDIVIDUAL ANTIBIOTICS

Penicillin. The mode of action of penicillin is in one sense the best known since it has been studied more fully than that of any other antibiotic; but, in another sense, it is not precisely known and indeed it is subject to considerable controversy and confusion. In part this appears to be due to the types of measurements which may be made. A particular and singular reaction appears to be blocked by penicillin. When it is so inhibited one may measure the lack of end products of this reaction or the accumulation of intermediates whose further metabolism would normally proceed through this reaction. While at present the nature of this reaction is difficult to specify in detail, its existence may be inferred from the relatively specific adsorption of penicillin to susceptible cells. After some initial confusion, in which claims were made for the absorption of penicillin by cell cytoplasm in quantities of less than ten molecules per cell,[7] separate groups of workers are now agreed[8–10] that there is a specific reversible uptake of penicillin most probably responsible for its antibacterial activity and that the component responsible for the adsorption appears to be located in the cell wall.[11] Once this penicillin binding component is inactivated, certain changes occur in the organism. These apparently have to do with disorganization in the metabolism (both synthesis and breakdown) of

nucleic acids, which in turn is related to the organism's ability to synthesize protein.[12] The effects on amino acid assimilation[13] and protein synthesis[14] are now thought to be reflections of the effect on nucleic acid synthesis.[12,13,15] Furthermore, several groups of workers have noted effects of penicillin (in relatively high concentrations) upon the nucleic acid metabolism of resting cells, and growing cells treated with penicillin show a relatively marked alteration in nucleic acid metabolism.[13,15–18] In addition, growing cells treated with penicillin show the accumulation of uridine-5′-pyrophosphates[19,20] whose quantities and kinetic relationships are such that they appear to be on the pathway toward nucleic acids, rather than related to any coenzyme function they might possess. There is thus an area of agreement among investigators that penicillin acts by inhibiting an early stage of nucleic acid synthesis (especially that of ribose nucleic acid) but it is as yet impossible precisely to pinpoint the site of action or to specify the exact mechanism of inhibition.

In spite of this area of agreement, it seems to be most difficult to penetrate further toward a solution of this mystery. There are a few observations (such as, in a gram-negative organism penicillin inhibits the growth when glycine is supplied but not when leucylglycine is available[21]) which do not fit into the present picture, and while the real significance of these "exceptions" remains uncertain, their existence means that there is still a great deal that requires careful study.

Since there are only indirect methods of measuring the site of action of penicillin and these do not lend themselves to studies in animal tissues, there is no experimental approach to the larger problem that now looms ahead. That is, why can penicillin be used in the animal body without untoward effects? Certainly this is well established; but does not the body synthesize nucleic acid and if penicillin stops this process in the gram-positive and certain other bacteria, why does it not do so in the gram-negative bacteria or in the animal cell? A variety of hypotheses can be proposed but to the best of our knowledge there is no experimental approach yet available for testing any of them.

Streptomycin. Streptomycin represents something of a contrast to penicillin in that a variety of reactions are inhibited by it. Streptomycin forms complexes with nucleic acids and nucleoproteins,[22,23] combinations which alter the surface charge of the bacteria;[24] it inhibits in a somewhat specific manner diamine oxidase,[25] an enzyme also inhibited by other agents; it interferes with inositol metabolism[26] and pantothenate synthesis,[27] and inhibits an unknown reaction called the "oxalacetate-pyruvate" reaction.[28,29] Because of the multiplicity of these effects it has been necessary to attempt to distinguish between those which might be related to the inhibition of the growth of the organism and those which might be related to the chemical properties of the molecule rather than to the antibacterial effect *per se*. The status of each of the reactions mentioned and their relation to possible mode of action have been reviewed elsewhere.[30] After application of the criteria mentioned at the start of this review, so far only one reaction survives as bearing a possible relation to the mode of bactericidal action. This is the "oxalacetate-pyruvate" reaction. Its nature remains somewhat obscure since it does not appear to be any of the known reactions of oxalacetate and pyruvate.[31] Inasmuch as reactions of these substances have been intensively studied, it would seem unlikely that further reactions involving them would be of quantitative significance. However, this appears to be the case. Some progress has been made in clarifying the nature of the streptomycin-sensitive reaction with the discovery of a new intermediate in metabolism, 2-phospho-4-hydroxy-4-carboxy adipic acid.[32] This compound is a seven-carbon phosphorylated tricarboxy acid, originally isolated from dog liver. It was shown to be an intermediate in the metabolism of the rat by tracing the incorporation of radioactive phosphorus into it. In Escherichia coli it is formed apparently only when a dicarboxy acid and pyruvate are present, and its formation is inhibited by streptomycin at levels comparable to those required to inhibit growth. However, it is not yet known what role this substance may play in metabolism. The site of action of streptomycin is thus biochemically pinpointed and, as in the case of penicillin, it turns out to be a relatively singular reaction essentially undetected by other methods of studying metabolism.

While all of this would seem to present a reasonable picture of the action of streptomycin one should point out that neither the "oxalacetate-pyruvate" reaction nor the new intermediate are particularly easy to measure, and there appears to be a considerable variation from strain to strain and with varied cultural

conditions. Thus much more remains to be done but, as with penicillin, there are no particularly promising tools with which to obtain more information than we already possess.

One seemingly fruitful approach toward a knowledge of mode of action is not applicable. It might be supposed that if one carried out comparable studies on bacterial strains which were sensitive to the antibiotic and those of its daughters rendered resistant, one might obtain a clue as to what the antibiotic was doing. This might be the case if various resistant strains were similar. But various streptomycin-resistant bacterial strains derived from the same sensitive parent show such a variety of alterations in metabolism, which are so inconsistent from strain to strain, that it is evidently impossible to provide any general explanation of the reaction inhibited by the sensitive strain.

The reasons why streptomycin may be used in the animal body are reasonably clear. The reaction sensitive to streptomycin occurs in animal tissues but a permeability barrier to streptomycin exists, not only at the cell wall but also at the surface of the mitochondria.[33] Pharmacologic studies show that while streptomycin does penetrate from the blood stream into the tissue, the amount so penetrating is very small. More direct studies of such penetration show that the cell is protected by an additional permeability barrier at the surface of the mitochondria, which is the apparent site of the sensitive reaction. This simple mechanism, that is, mere physical separation of streptomycin from the site of the sensitive reaction, seems to account for its ability to kill those bacteria which it can attack in the animal body. This means, however, that if the sensitive bacteria are protected from the streptomycin by themselves growing within the host cell, or walled off in other ways, they will not be attacked by the drug, which apparently explains the usual failure of streptomycin in brucellosis.[34] This factor of penetration also plays an important role in the treatment of tuberculosis.[35]

Chloramphenicol. The mode of action of this substance is essentially unknown. It does not inhibit a wide array of enzyme-catalyzed reactions.[36] In concentrations about tenfold higher than those required to stop growth, it inhibits bacterial esterase.[37] In growth studies its action is decreased by the presence of phenylalanine, tyrosine or tryptophan,[38–40] which suggests interference with the formation or metabolism of these amino acids but the effect is demonstrable only over a narrow range. Other data[41–42] indicate that this effect cannot be the mode of action. Naturally, the reasons why it may be used in the animal are even more obscure.

The Tetracyclene Group. There are several indications that aureomycin and terramycin do not act in the same manner,[3] but these could well arise from differences in absorption or penetration so that, in view of the close similarity of structure, one can best regard their mode of action as similar if not identical. At rather high levels in animal tissues they inhibit aerobic phosphorylation either because of inhibition of some part of the Krebs cycle of respiration or vice versa.[43,44] Unfortunately, this particular action seems to be relatively non-specific in that various other substances, some having no antibiotic effect, possess the same property.[3] Furthermore, while the same effect may be noted in bacteria, growth and protein synthesis are inhibited by much lower concentrations of the drugs.[12,41] A cell-free nitrate-reductase preparation was inhibited by aureomycin but this was apparently due to combinations with Mn^{++}.[45] The actual mode of action of these substances is still unknown.

Other Antibiotics. It happens that information on the mode of action occasionally is available for a particular antibiotic even though it may be of little clinical significance. Most of these are listed here, primarily since they serve to point out that there is no one mode of action for all the antibiotics. Some antibiotics, tyrocidin[46] or subtilin[47] for example, are surface-active agents and may be regarded as altering the physical structure of the cell. Antimycin A appears specifically to inhibit one step in a complex respiratory chain.[48] Ballomycin B forms a complex with the respiratory pigment, cytochrome c, and this is possibly its mode of action.[49] Actithiazic acid appears to interfere with the synthesis of biotin.[50] Polymyxin seems to combine with polyphosphates within the cell.[51]

SUMMARY

It seems apparent from even this brief review that various antibiotics have varied modes of action, although the mode of action of the majority of them is not known. However, for the major antibiotics (penicillin, streptomycin, chloramphenicol and the tetracyclenes) it is known that they interfere, apparently irreversibly, with some important biochemical

reaction in the cell, and that this reaction differs with each antibiotic.

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Bacterial Resistance*

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FEW tasks are more difficult than attempts to illuminate the present in the light of a controversial past. Yet this is the usual prospect in a consideration of problems arising from the study of bacterial resistance to antibiotics and other drugs. Clinical and experimental information available in the literature shows that even simple principles concerning the nature of drug resistance have been slow to emerge, and many findings have been derived empirically. For example, what kind of knowledge would have enabled investigators to predict that penicillin resistance would become a serious problem in the treatment of staphylococcal infections but of little significance in diseases caused by Streptococcus pyogenes?[1,87] How does one explain the apparent failure of *p*-aminosalicylic acid to retard the development of streptomycin resistance in mice but not in humans?[88] Why do some isoniazid-resistant mutants of Mycobacterium tuberculosis retain their virulence for mice but not for guinea pigs?[23] What is the basis for the frequent emerging of streptomycin dependence,[64] as contrasted with the rare and transitory quality of dependence on penicillin,[7] chloramphenicol[77] or isoniazid?[14]

On the other hand, the existence of numerous unexpected and unsolved problems need not obscure the fact that a great deal is now known about the origin and nature of bacterial resistance to drugs. Since a large proportion of drug-resistant variants appear to arise by mutation, the origin of resistance is a legitimate field of study for genetics, although it is not exclusively within this province. Mechanisms of resistance provide material for the biochemist and bacterial physiologist. In the final analysis only the physician can evaluate the medical significance of drug-resistant bacteria.

Several reviews are available that discuss the development of microbial drug resistance in technical detail and at considerable length.[1-21] In the following paragraphs an attempt will be made to formulate and analyze problems involving the origin and nature of bacterial resistance to antibiotics, exclusive of mechanisms. Emphasis will be placed on general features of interest to physicians unfamiliar with the specialized vocabulary and other esoterics employed by microbiologists and bacterial geneticists.

Prevalence and Evolutionary Significance of Antibiotic Resistance in Bacteria. There are many chemical agents which produce irreversible cellular damage and against which the development of any substantial degree of resistance is improbable. From this standpoint such agents may seem of potential value in chemotherapy. At a more comprehensive level chemical substances of relatively uniform and universal cytotoxic action are at once excluded as useful antibacterial drugs. Concentrations effective for chemotherapy may also be damaging to the host tissue. Certain other chemical substances, including the medically practical antibiotics, are characterized at therapeutic dosage levels by a more or less restricted action on specific microorganisms. It may be assumed that agents acting only on special classes of cells do so primarily by interfering with the subtle details of biochemical processes which set these cells apart from others that are similar but not identical. An effective antibiotic may block a specific enzyme; it must not act on enzymes generally, as by denaturation.

It is the very specificity of antibiotic action that permits evolutionary diversity to give rise to resistant strains. Minor variations of respiration or biosynthesis, as a constant product of mutation, will lead to the origin of new types of organisms. The new varieties will possess systems of metabolism almost like the parent strain, since evolution characteristically pro-

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ceeds in small steps. Drug-resistant bacteria may result when the genetic change provides an alternative or more efficient method for synthesizing an antagonized essential metabolite.[12] Other mechanisms of resistance include decreased affinity of the drug for cellular components,[36] and synthesis of drug-inactivating enzymes.[2,63] But since the ability of the bacterial cell to adapt physiologically or to produce new variants of a resistant nature is itself a hereditable property, the final arbiter of resistance is inherent in the genetic constitution of the bacterial cell. The development of resistance to streptomycin or penicillin in a sensitive strain is therefore in no way remarkable as an evolutionary change, since the property of resistance is only an incidental by-product of small biochemical alterations, predominantly of mutational origin. Presumably, these same mutations have been occurring for millions of years, but with an adaptive value insufficient to establish the resistant strains as successful evolutionary types except in recent periods when the natural environment has been disturbed by the wide prevalence of antibiotics.

The occurrence of antibiotic-resistant bacteria is thus a consequence of evolutionary pressures leading to the selection of types best fitted for survival. The absence of drugs in the natural environment puts no evident selective advantage on drug resistance as a hereditary trait in naturally resistant species. Obscure hidden advantages may exist or the property may be selectively neutral. In sensitive species the resistant derivatives arising as mutants are outnumbered and at a selective disadvantage in the absence of the drug; otherwise they would themselves comprise the "wild type" or possibly a so-called "naturally" resistant strain. When different kinds of drug-resistant mutants arise, some may tend to be selectively favored in the human host. Thus penicillinase-producing staphylococci are usually but not always found to the exclusion of non-penicillinase producers in pyogenic infections.[10,74] In the laboratory mutants that resist penicillin without dependence on the extracellular inactivation of the antibiotic are isolated in great excess, but again not exclusively.[72,78] The difference is probably selective (that is, secondary) and therefore quantitative rather than qualitative.[78] An exploration of the diversity of penicillin-resistant staphylococci has been made by Eagle.[36]

Two major classifications of bacteria may be considered. Some species or strains appear to lack entirely the antibiotic-sensitive substrate affected by a particular drug, perhaps possessing a compensating or insulating mechanism. The clinician would not ordinarily treat a proteus infection with penicillin, nor would he employ bacitracin in the treatment of a disease caused by coliforms. At the other end of the scale are bacteria existing as sensitive species or strains and commonly giving rise to resistant mutants. In reality the designation of bacteria as resistant or sensitive is complicated by the relative and continuous nature of the classification.

In Table 1 four representative species of bacteria, tested by a single method (gradient plate),[76] have been classified with reference to antibiotic or drug sensitivity.[77,79,82,84] The agents in each column are arranged in order of decreasing effectiveness against the organism listed at the head of the column. It is clear, for example, that Micrococcus pyogenes is sensitive to penicillin and relatively resistant to nicotinaldehyde thiosemicarbazone. Response to the intermediate drugs permits the classification of micrococcus as either sensitive or resistant, depending on the standards of comparison. The problem is hardly simplified by wide variations in the sensitivity of different naturally occurring strains, and the evident complications encountered *in vivo*. Iproniazid and PAS, of comparatively low potency against an acid-fast saprophyte (Micrococcus ranae) are highly active in preventing the growth of Mycobacterium tuberculosis. Ultimately, the solubility or therapeutic index of a drug may determine its choice, not the resistance level of the pathogen.

It is convenient to speak of mutation followed by selection as evolutionary adaptation and of drug-induced resistance as physiologic adaptation.[75] Ability to undergo a particular physiologic adaptation could arise (or be lost) at any time during the course of evolutionary adaptation. The demonstration that resistance commonly arises by mutation-selection does not exclude the possibility that under specific experimental or clinical situations another method of resistance development may be operative. Numerous polemics have surrounded attempts to decide whether (or more accurately, *when*) drug resistance is induced or selected in the pathogen. Attention will first be directed to a brief survey of patterns of bacterial drug resistance with special reference

to antibiotics and with minimum regard to examples of problematic status.

ANALYSIS OF RESISTANCE PATTERNS

The Penicillin Pattern. Soon after the introduction of sulfonamides and penicillin as chemotherapeutic agents it was reported that resistant strains of bacteria might seriously reduce the efficiency of these drugs.[3,61] Collaborators of Ehrlich had noted the emergence of arsenic fastness in trypanosomes, and the problem of drug resistance undoubtedly originated in the historic infancy of chemotherapy and will continue to attend its maturity.[89] A characteristic feature of resistance development in the presence of most chemical agents is the gradual and apparently continuous or step-wise nature of transitions from sensitivity to the upper limit of resistance. Numerous intermediate grades may be isolated during the experimental procedures required to produce fully resistant organisms.

An analysis of the transitional process is provided by a determination of the resistance pattern. If penicillin-sensitive staphylococci are grown to sufficient titer to include a variety of genetic types, the resistant component can easily be isolated by plating samples of the bacterial population on nutrient agar media containing graded concentrations of the antibiotic.[27] By plotting numbers of survivors against the rising drug concentration one obtains a descending curve which indicates survival from 100 per cent to zero. These experiments with M. pyogenes showed that no bacteria survived a penicillin concentration of .15 units per ml. Colonies surviving slightly lower concentrations, however, proved to be composed of first-step resistant mutants, able upon further cultivation and testing to produce populations containing some individuals that survived higher concentrations of penicillin, with ultimate inhibition of all cells at 0.2 units

TABLE 1*

Escherichia coli		Micrococcus pyogenes		Mycobacterium ranae		Bacillus megatherium	
Drug	μg./ml.	Drug	μg./ml.	Drug	μg./ml.	Drug	μg./ml.
Bacitracin	700	Nicotinaldehyde thiosemicarbazone	1500	Nisin	3000	Nicotinaldehyde thiosemicarbazone	800
Circulin	60	*p*-Aminosalicylic acid	500	PAS†	3000	Isoniazid	400
Viomycin	50	Nisin	500	Amithiozone†	1500	*p*-Aminosalicylate	400
Polymyxin B	20	Vinactin	200	Iproniazid†	1500	Penicillin	20
Netropsin	8	Circulin	120	Penicillin	900	Pleuromutilin	10
Catenulin	6	Viomycin	80	Bacitracin	200	Illudin-M	10
Penicillin	5	Polymyxin B	60	Patulin	30	Vinactin	10
Streptothricin	4	Licheniformin B	50	Polymyxin B	25	Viomycin	10
Neomycin	2	Bacitracin	20	Chloramphenicol	20	Streptothricin	8
Streptomycin	2	Netropsin	10	Streptothricin	15	Circulin	6
Aureomycin	1	Mycomycetin	10	Thiolutin	15	Furadroxyl	6
Terramycin	1	Patulin	6	Illudin-S	5	Patulin	5
Chloromycetin	1	Tyrocidin	5	Isoniazid	5	Mycomycetin	3
		Streptothricin	4	Netropsin	4	Polymyxin B	1.5
		Chloramphenicol	4	Vinactin	4	Tetracycline	1.5
		Streptomycin	3	Viomycin	2	Carbomycin	0.8
		Thiolutin	3	Catenulin	2	Terramycin	0.8
		Subtilin	2	Illudin-M	1	Streptomycin	0.7
		Catenulin	1	Streptomycin	0.6	Tyrocidine	0.5
		Neomycin	0.8	Neomycin	0.5	Subtilin	0.2
		Terramycin	0.5	Terramycin	0.4	Erythromycin	0.2
		Aureomycin	0.3	Mycomycetin	0.2	Bacitracin	0.05
		Penicillin	0.02	Aureomycin	0.05	Neomycin	0.04

* Approximate concentration in micrograms per milliliter required to produce detectable inhibition of cells in streaks of representative bacterial species on gradient plates.[77,79,82,84]

† Active against M. tuberculosis.

per ml. These second-step mutants gave rise to variants which were not completely eliminated until the penicillin concentration reached 0.4 units per ml. The degree of sensitivity now decreased rapidly with each step, suggesting an interaction of genetic factors for resistance that was more than additive. After five consecutive isolations of mutants, having progressively greater resistance and presumably corresponding larger numbers of genes affording protection against the drug, it was finally impossible to obtain complete bacteriostasis of cultures at penicillin concentrations of 250 units per ml. Successive resistance curves revealed clearly this tendency of each newly isolated mutant to produce cultures containing a higher proportion of resistant individuals and having a new upper limit of complete inhibition. The complex of curves so obtained constitutes the penicillin pattern. Most antibiotics, acting on sensitive strains, elicit the same gradually increasing progress of resistance development.[15,29]

Certain consequences are inherent in the penicillin or obligatory multistep pattern as it relates to the establishment of full resistance. Assuming an originally sensitive strain, full resistance will not be possible without the intervention of several intermediate bacterial populations of large size. Since most mutations are rare events, each progressive step requires the prior multiplication of an antecedant type in numbers sufficient to favor additional mutation on a probability basis. Furthermore, the use of high concentrations of penicillin at the outset should completely prevent the secondary establishment of highly resistant strains.

The Streptomycin Pattern. Resistance to streptomycin,[29,30] PAS[83] and isoniazid[81,83] develops according to a different kind of pattern, or rather a variety of patterns. The possibility of isolating numerous mutants with a low-grade resistance,[46,65] and from these obtaining further steps in resistance development, suggests that streptomycin may accumulate small mutations in the same manner as penicillin. However, there exists a class of mutants, arising in the growth of sensitive cultures, that are not inhibited by any therapeutically practical concentration of drugs defined by the streptomycin pattern. Stated in somewhat different terms, it appears that mutants with many grades of resistance can arise in one step. Interaction of numerous minor genes for resistance is evidently not required, a single mutation sufficing to give comparatively great protection against streptomycin, isoniazid or PAS (facultative one-step resistance). If grown to sufficient size, predominantly sensitive populations of bacteria will always contain small numbers of cells which are able to form colonies in agar containing relatively high concentrations of these antibacterial agents. A larger class of intermediate mutants is at the same time eliminated at high concentrations.

Intermediate Patterns. In normal strains of Escherichia coli the rate of mutation to polymyxin resistance is fairly low. Nevertheless, in sensitive cultures exceeding 2×10^9 cells, clones may be found that remain viable and form large colonies at concentration in nutrient agar of 60 μg. per ml. These survivors if subcultured may give rise to mutants with increased resistance.

Clinical Implications of Resistance Pattern. Evidence obtained in the laboratory can serve merely as a guide to the clinician. Among the useful laboratory tests applicable to new antibiotics and drugs is a determination of resistance pattern, with appropriate recommendation of multiple chemotherapy if warranted by experimental findings. The fact that drug resistance has been of great significance in the therapy of tuberculosis is undoubtedly related to a peculiar coincidence easily shown by experiments, namely, that full resistance to streptomycin, isoniazid or PAS can arise by a one-step change. In contrast, development of penicillin resistance in staphylococci as a widespread phenomenon has required years instead of months; the obligatory multistep pattern does not permit the rapid emergence of resistant strains.

Although resistance patterns are important in predicting the value of chemotherapeutic agents, other factors undoubtedly influence the establishment of resistant bacteria in human individuals and ultimately in human populations. Before resistant strains can become manifest some change must occur in certain organisms descended from sensitive parental types. How does antibiotic resistance arise in cultures of growing bacteria after inoculation of a few sensitive cells into nutrient medium?

GENETIC FOUNDATIONS OF ANTIBIOTIC RESISTANCE IN BACTERIA

Clonal Relationship of Resistant Cells. In considering the nature of bacterial resistance to antibiotics, some reference should be made to

the significance of clones. A clone may be defined as a group of organisms descended asexually from a single individual. Most experimental demonstrations of the spontaneous origin of drug resistance depend on methods for detecting the presence of one or more antibiotic-resistant clones in cultures originating through the growth of a few sensitive cells. An essential part of the demonstration is to prove that the clones were already in existence at the time the drug was applied and hence could not have been induced. For example, let us suppose that a culture started from a single streptomycin-sensitive cell has grown to a titer of 2×10^{11} cells and contains, as shown by plating on streptomycin-agar, a total of sixty-four resistant colony-forming organisms. It might be considered that the sixty-four resistant cells were induced at once by contact with the drug. On the other hand, a single mutant arising six generations previously could have produced the sixty-four resistant cells as a clone (that is, 1, 2, 4, 8 and so on). Allowing one-half hour for division, the original mutant must have appeared over three hours before its descendants came in contact with the drug. In reality a considerable range of statistical fluctuation applies to the time of appearance of the first mutant; cultures at a later stage may contain several small clones mixed together with new mutants constantly arising. The statistical fluctuation allows isolation and identification of the clone in time of origin with a variance suggesting relation by descent.[27,60] A replica-plating technic devised by the Lederbergs[57] permits isolation and identification of the clone in space, since on solid medium mutant cells and their descendants will accumulate in isolated locations on a drug-free Petri dish. "Printing" an impression of bacteria covering the drug-free plate on to another containing the drug will allow localization and later concentration of the original drug-resistant clones. The question of whether members of such a clone require drug contact for the final transition from potential to actual resistance is amenable to test but is of little more than academic interest.

Accumulation of mutant cells in continuous culture devices, as shown by Novick and Szilard[68] and Bryson,[18] is one of several other ways of proving the spontaneous origin of bacterial clones resistant to harmful agents. The demonstration that clones are composed of genetic mutants can only be inferred unless other tests are performed. More convincing evidence of a chromosomal or genic basis for antibiotic resistance can be made only with other types of experiments, notably transformation, transduction and recombination. Severe limitations with respect to the number of bacterial species that are suitable for the performance of these specialized types of experiment have resulted in the extension of rather meager findings to a wide variety of microorganisms. Only the future can show whether the extrapolation has been justified.

Transformation. If genes are composed of (or invested by) deoxyribonucleic acid (DNA), it would appear feasible to prepare a gene "soup" by purifying this chemical, freeing it from conjugated protein without depolymerization. Bacteria immersed in purified DNA from other strains do apparently become infected with new hereditary properties, as originally shown for pneumococcal type transformation by Avery, MacLeod and McCarty[6] in experiments based on the earlier observations of Griffith.[42] Hotchkiss[45] and Alexander and Leidy[5] are currently studying the transformation of antibiotic resistance in the pneumococcus and Hemophilus influenzae. In agreement with the modern concept that carriers of heredity–genes are chemical substances, transformation demonstrates that antibiotic resistance can be transferred from resistant to sensitive cells by extracellular contact with chemical fragments of hereditary material. The extracellular genetic information is incorporated, assimilated and replicated at cell division in a few competent bacteria. The transforming agent cannot carry over resistance factors unless they are already in existence in the donor strain. Hence the process tells nothing about the origin of antibiotic resistance but does provide additional evidence that resistance may have a genetic basis.

Transduction. Another form of evidence for the genetic nature of antibiotic resistance may eventually be obtained through studies of transduction. In simple terms, transduction occurs when a bacteriophage serves as a vector transferring genetic properties from one bacterial cell to another.[90] The recipient cells must belong to a lysogenic strain, living symbiotically with the phage. It appears that bacteriophage may escape from a lysogenic strain and enter a sensitive strain, wherein reproduction is followed by lysis of the sensitive bacterial host.

Returning to their original lysogenic host strain, they re-enter and bring small pieces of genetic material from their temporary abode in the sensitive strain. If the sensitive strain carries genes that the lysogenic strain did not originally possess, a small proportion of the recipient symbionts are transduced, that is, acquire foreign genes from the lysed strain. Restorations of nutritional deficiencies are easily brought about by transduction. Zinder and Lederberg have reported transfer of streptomycin resistance by transduction,[90] although difficulty is now encountered in attempts to repeat the experiment. Attempts by Bryson to transduce sensitive cells to polymyxin resistance have not succeeded, nor has Hartmann been able to transduce cells to azide resistance (unpublished). More work is evidently required before the transduction technic can be used successfully in the study of antibiotic resistance; the difficulties may prove to be technical and temporary. A potential application is the use of simultaneous "linked" transductions to determine the linear order of genes in bacterial species that have no known sexual phase.[34] So far, the most informative experiments on the nature of antibiotic resistance have been studies of recombination.

Bacterial Recombination. By means of ingenious experiments, wherein nutritionally deficient strains of Escherichia coli were mixed and plated on synthetic media, Tatum and Lederberg were able to confirm the long-suspected existence of sexuality in bacteria.[85] These experiments have since been extended by Lederberg and numerous other investigators to include several strains[55,58] and at least one other species.[8] The experimental material is marked by certain unique peculiarities. The proportion of nutritionally inviable bacteria that "mate" and give rise to viable individuals may be only a few dozen out of many millions placed together for crossing purposes. The life of the average bacterium is not without an element of frustration. As a second distinction, ordinary strains of bacteria except at division contain only a single set of chromosomal elements per nucleus, with the fusion and doubling process of fertilization existing momentarily. Dominance and recessiveness can therefore be detected only in aberrant diploid bacterial strains containing two sets of "chromosomes" in each nucleus. Such diploid strains have served to establish that streptomycin resistance is a genetically recessive trait.[56] The introduction of unlike hereditary determinants (from genetically dissimilar parental types) into a temporary fusion relationship permits genetic recombination. In other words, chromosomal elements entering the cross as ABCD and abcd may emerge recombined as ABCd and abcD as a result of crossing over. In E. coli one parent may not contribute a full set of genes. The essential point is that, as in higher plants and animals, the genes of bacteria may be arranged in a linear order and precisely localized on the bacterial equivalent of chromosomes.[58] The gene, or gene system, for streptomycin resistance lies adjacent to a gene for maltose fermentation. The multifactorial nature of high chloramphenicol and terramycin resistance is confirmed by the random location of several independent genes for resistance to these antibiotics.[20] Recombination has removed the genetic basis for resistance to some antibiotics from the realm of speculation. There is no reason to assume that, in bacteria having no detectable sexual process, resistance to the same agents used at the same concentration will depend on non-genetic factors. Similarly, experiments conducted at an earlier date are entirely consistent with an interpretation which identifies resistance developing in "steps" as multifactorial (penicillin pattern) and one-step resistance as the marked effect of a single mutation at one locus (streptomycin pattern). An understanding of these problems will be aided by attention to the nature of mutation.

NATURE OF SPONTANEOUS AND INDUCED MUTATION

Mutation Rates. The rate of mutation to streptomycin resistance has been measured more frequently than that of any other genetic change leading to bacterial survival in a toxic environment. As summarized by Braun,[11] this rate is close to 10^{-10}. If a single sensitive bacterial cell is permitted to divide until a large population is formed, the mutation rate will ensure on the average that one resistant cell will appear when the population size is 10^{10} cells (reciprocal of 10^{-10}). The proportion of resistant cells will then increase rapidly, since each mutant by definition must on division simultaneously produce two individuals like itself, except in the rare case of reversion. Additional streptomycin-resistant cells will arise, together with miscellaneous mutations affecting ability to perform various steps in biosynthesis and inci-

dentally affecting resistance to other drugs. A culture descended from a single cell is thus by no means pure genetically.

It is believed that frequency of mutation is related to the molecular configuration of genes. According to one theory, when cells divide the genes are duplicated and the more unstable genes are prone occasionally to imperfect replication, resulting in an altered molecular structure. Under special circumstances the rate of mutation can be experimentally accelerated, as when cells are exposed to x-rays, gamma rays, nitrogen mustard and other so-called mutagenic agents. Since many chemicals are mutagenic,[33] it is of interest to know whether antibiotics are included among substances capable of inducing genetic change either in bacteria or in the reproductive cells of the host. As far as we are aware, no highly mutagenic antibiotic is known, although some may perhaps induce bacterial mutations non-specifically.[66] Recently Akiba[4] reported a possible inductive effect leading to drug resistance. This work of Akiba has been repeated with somewhat indefinite results by Szybalski.[80]

The experiments of Akiba require further analysis. In general, no chemical or physical agent can induce drug resistance except by direct effect on the chemical constitution of genetic materials in the nucleus of the cell. Many antibiotics have extreme non-genetic effects on structures seen within bacterial nuclei.[26] These structures resemble the chromosomes of higher organisms although the exact degree of homology is a moot question.[25] The site of action for genetic change is believed by many geneticists to be deoxyribonucleic acid (DNA), a major component of chromosomes and the putative matrix or chemical counterpart of the gene. The inability of most environmental agents to disturb the stable structure of genetic determinants, with ensuing adaptive advantage in relationships with the disturbing agent, accounts for the present discredited position of Lamarck's theory. The failure of most if not all antibiotics to induce major genetic resistance in a restricted and specific sense is part of the same problem. Minor physiologic adaptation in direct response to drug contact is not a pertinent part of a discussion limited to genetic considerations.

An interesting development in the study of antibiotic resistance is the discovery of a gene in E. coli that increases the mutation rates of all other loci (excepting one).[86] Genes increasing spontaneous rates of mutation are also known in higher plants[62] and animals.[48] The mutable strain may be crossed to a non-mutable parent type, allowing segregation of the locus for mutability. Experiments by Treffers et al. on the rate of mutation to streptomycin resistance in the mutable E. coli show about a hundredfold increase in the frequency of resistance development. If mutability genes tend to be selected by a plethora of antibiotic agents, the role of drug resistance in therapy may suddenly become much more important.

Specificity of Mutations to Resistance. It has already been stated that apparently there exist numerous interactive genes which control resistance to either penicillin,[27] chloramphenicol or terramycin®.[21] Also the presence of one-step mutants with different levels of streptomycin resistance, including the group of dependent strains, clearly implies a variety of types. Streptomycin-dependent mutants of M. ranae can be subdivided into an almost continuous spectrum by quantitative determinations of the degree of dependency,[47] and the non-dependent strains of E. coli can usually be distinguished by careful observation of colonial morphology on MacConkey medium, or by studies of other secondary properties.[11] If the mutations to full streptomycin resistance and dependence occur at one genetic locus, a large system of multiple alleles appears to be involved.[31,67] Many antibiotic-resistant strains characteristically differ in some manner from other strains isolated at the same drug level and differ more markedly from the strains isolated at other levels. Particularly during the selection of mycobacteria resistant to very high concentrations of isoniazid, one obtains aberrant mutants differing in minor biochemical properties from each other[24] and in colonial morphology from the parent strain.[14]

The wide diversity of mutant types leads to several considerations. The assumption that antibiotic-resistant strains isolated in different hospitals or laboratories are identical may be a delusion. Similarities will exist, but there will usually be minor differences which will tend to be disregarded if they have no clinical significance. The important point is that generalizations about the properties of resistant strains will be based on collective observations of a non-homogeneous group. It may indeed be true that resistance to a certain antibiotic or drug tends to reduce the pathogenicity and virulence of a particular organism, in most instances. But the

potential exception to the rule, endowed with greater evolutionary advantage, may eventually show that complete trust in a standard description of resistant strains is not a safe clinical procedure. The use of an antibacterial agent singly, with the rapid development of a resistant but comparatively avirulent population of cells, cannot be relied upon as a safe program of therapy unless the possible emergence of exceptional mutants is taken into account. Dispersion of a resistant and unusually virulent mutant among the general human population would seriously impair the usefulness of the drug.

GAIN AND LOSS OF RESISTANCE IN BACTERIAL POPULATIONS

Establishment of Antibiotic Resistance in a Bacterial Population. The origin of populations of drug-resistant cells is a possible but by no means necessary consequence of mutations to drug resistance in single cells. Establishment of a mutant population will usually require positive selection. Now, it has already been suggested that resistant species are well adapted for survival in nature, whereas resistant strains with few exceptions are positively selected only in the presence of a particular drug and should tend to disappear when the drug is no longer used. Hopps, Wisseman and Whelan have shown that the frequency of antibiotic resistance is relatively low in regions of the world having poorer standards of medical care, an observation consistent with the concept of positive selection as a factor for increased drug resistance in our own medically advanced society.[44] A neglected factor in estimating the future of resistant strains is the effect of selection on the resistant-population size and hence on opportunity for further mutation. For example, in the absence of streptomycin, cells resistant to this antibiotic exist as a small minority of evolutionarily unsuccessful individuals submerged among vast numbers of sensitive cells. When streptomycin is present, the resistant population is given an opportunity to increase enormously at the expense of the sensitive parental type. Within the large resistant population there can now be selective competition among further mutants of rare types for general evolutionary fitness. In theory, the resistant strain might produce substrains, one of which could be equal in fitness to the sensitive parental type, even after the eventual removal of the drug. The tendency of resistant strains to appear and disappear must be considered in the light of a multitude of cellular properties, including virulence, growth rate, pathogenicity, longevity, invasiveness and numerous other traits influencing survival value. All are presumably or demonstrably subject to change by mutation.

Selective factors are at least as important as mutational factors in the establishment of resistant bacterial populations. The responses to selection go beyond those mediated by the mere presence or absence of a drug. Numerous recent studies on cross infection in hospitals show that antibiotic-resistant staphylococci are sufficiently successful to exist endemically among the professional personnel,[40,54,59,6,71] replacing the sensitive parental strains even in persons not receiving antibiotic therapy. These are probably "naturally" resistant strains rather than recent mutants. Replacement is not necessarily competitive but may occur when a resistant invader finds a temporary ecological vacancy.

Secondary Loss of Resistance. Microorganisms resistant to streptomycin but not requiring it for growth are notably viable and may be cultivated indefinitely in the laboratory with no evidence of resistance loss. However, loss of streptomycin resistance is not unknown.[9] Many other antibiotics (e.g., polymyxin) may select resistant strains that grow slowly even in the absence of the drug. Still others lead to the isolation of resistant populations that die after a few days of storage on nutrient agar at refrigerator temperatures. Obviously antibiotic resistance may be accompanied by a severe reduction in viability.

One means of secondary loss of antibiotic resistance in mutants with low viability is through additional genetic changes that lead to increased growth rate or viability and a corresponding reduction in resistance properties. No satisfactory method of screening for mutations from streptomycin resistance to streptomycin sensitivity is known, but mutations from dependence to sensitivity are easily observed in E. coli[32] and occur at rates of about 10^{-8}. Many genetic changes designated as "reversions," without restriction to drug resistance alone, are found to resemble the parent type but not to be identical with it.[31] Alternations between resistance and sensitivity in most instances should be represented as $S \rightarrow R \rightarrow S' \rightarrow R' \rightarrow S''$ and so on, not as $S \leftrightarrows R$. The loss of isoniazid resistance in M. ranae appears in some instances to result

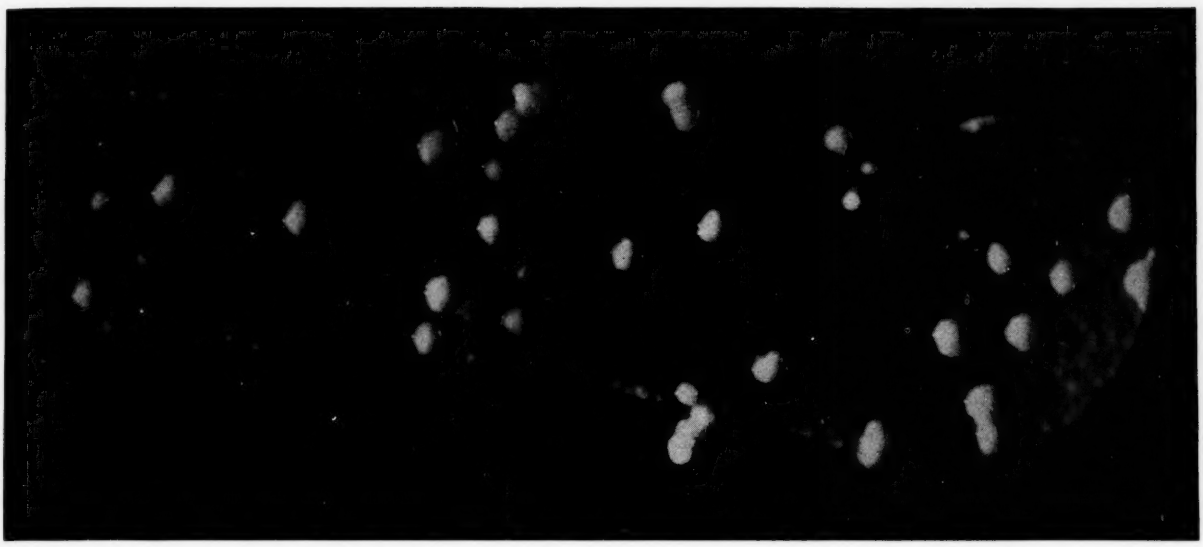

FIG. 1. A slow-growing strain of isoniazid-resistant Mycobacterium ranae plated in the absence of the drug. Mutations to faster growth rate produce separate clones evident as papillae. These clones may or may not be less resistant. Many isoniazid-resistant strains grow at the normal rate when isolated *in vitro* and the rate of papillae formation in slow growers depends on the individual strain and is characteristically lower than depicted in this example.

from additional "suppressor" mutations which destroy the resistance property but do not necessarily modify such accessory secondary characters as colonial morphology. However, unless crosses can be made the role of suppressors is speculative, and M. ranae cannot be mated.

Emergence of faster-growing mutants as papillae in a streak of isoniazid-resistant M. ranae is shown in Figure 1. Similar mutants appear on colonies grown from single cells prepared by agitating the growing culture continuously through forced aeration, using nutrient broth containing 0.1 per cent triton A-20 to prevent clumping. The peculiar morphology is characteristic of M. ranae isolated from very high concentrations of isoniazid (500 μg. per ml.). Tests often prove the faster-growing mutant derivatives to be more drug sensitive but are not necessarily so, each papilla or clone being composed of a specific type of cell. In the strain depicted in Figure 1 mutations toward isoniazid sensitivity retained the abnormal morphology. Other strains have been isolated in which revertants to sensitivity also reacquired the typical waxy appearance of the parent type. All cells were acid-fast at some stage during cultivation.

SIGNIFICANCE OF MULTIPLE CHEMOTHERAPY

There is general agreement that the indiscriminate use of antibiotics to which organisms show the streptomycin pattern of resistance is fraught with the risk of selecting resistant strains.[37] As a consequence, streptomycin is now routinely combined with PAS in the treatment of pulmonary tuberculosis, and almost every conceivable combination of antibiotics has been employed in chemotherapy. Evaluations of resultant synergism and antagonism have been made by numerous observers, notably Spicer,[73] Klein et al.[52,53] and Jawetz et al.[49–51] The prevention of resistance is evidently only one of the possible advantages of appropriate combinations of antibiotics.

In theory, combined drugs should select doubly resistant mutants that arise with a frequency determined by the product of the single mutation rates.[17,28] These single mutation rates tend to be approximately the same in different species of bacteria, perhaps representing changes of the same genetic locus in the bacterial chromosomes. It will be remembered that similar forms of life share a similar genetic constitution. We have already seen that during the growth of most bacterial cultures only about one cell in ten billion mutates to streptomycin resistance. Other tests show that about one cell in a million mutates to isoniazid resistance.[81] Obviously the theoretic chance that both mutations will occur in a single cell simultaneously is only 1/10,000,000 × 1/1,000,000,000, or 1/10,000,000,000,000,000. How, then, does one account for the appearance of doubly resistant strains in the laboratory at rates greater than

expectancy?[83] And how do populations of resistant cells arise eventually in many patients treated with multiple chemotherapy? Several factors may be involved.

First, it is established that when the size of a bacterial culture exceeds the reciprocal of the mutation rate, the proportion of mutants will increase rapidly, particularly if the mutants are not retarded in growth.

Mutants		*Approx. Total Cells*	*Ratio Mutants/Non-mutants*
New	*Total*		
1	1	10^{10}	$1/10^{10}$
2	2 + 2 = 4	2×10^{10}	$2/10^{10}$
4	4 + 4 + 4 = 12	4×10^{10}	$3/10^{10}$
8	8 + 8 + 8 + 8 = 32	8×10^{10}	$4/10^{10}$
	clones		

This is because, once a mutation to resistance has occurred, the resistant cell on division will produce two resistant daughter cells; these in turn produce *four*, and so on, resulting in the formation of clones, as already shown. At the same time, new mutations are taking place as the bacterial population enlarges. This proportional increase of mutants is significant, because therapy is customarily administered after the patient has shown evidence of clinical symptoms and may already harbor a large population of cells, perhaps enough for some doubly resistant forms to be present. It should be recalled that in the laboratory it is not difficult to grow bacterial cultures containing 10^{9} cells per cc. Yet this number of cells seems very large for most types of infection and it is uncertain whether under non-selective conditions (that is, before drug treatment) mutant clones would attain sufficient size to permit additional mutations to occur within them.

Another factor of possible significance in reducing the efficiency of combination chemotherapy is the production of induced multiple mutations as a secondary consequence of some drug-conditioned disturbance of bacterial metabolism. Double mutants occur very commonly in bacteria as a result of exposure to certain toxic physical or chemical mutagens[13,16] but the extension of these findings into the realm of non-specifically induced mutations to drug resistance must await further study. An additional potential handicap of any chemotherapy is the possible isolation of pathogens in the body, with resulting ineffective concentrations of drugs, a serious possibility in preliminary phases of treatment. Mixed drugs may have unequal partition coefficients in living tissue. Even bacteria in contact with antibiotics could be isolated physiologically. For example, E. coli that are not actively metabolizing may be temporarily immune from the effect of an antibiotic.[43,70] Spurious "isolation" by antagonistic combinations of antibiotics is of utmost importance.[51]

A further consideration in the development of double or multiple resistance pertains to our frequently inadequate knowledge of the potential interaction of genetic factors. As previously observed, the development of marked antibiotic resistance may be based on the cumulative effect of numerous small independent mutations, either presumptive[27] or demonstrable.[22] The emergence *in vivo* of bacterial strains resistant to two agents administered simultaneously may then depend on the characteristically higher frequency of mutations to a low level of resistance, coupled with rapid development of complete resistance from the intermediate stages through physiologic interaction and the accumulation of additional mutations. The demonstration by Demerec, that penicillin resistance develops by a series of steps with exponentially increasing effectiveness,[27] together with Cavalli and Maccacarro's observations of chloramphenicol resistance as related to certain combinations of genes,[22] raises a significant possibility. Once a low degree of resistance has been established, either directly or by cross resistance, the onset of higher resistance may ensue very rapidly through the combination of certain "resistance" genes, the effects of which are more than additive. The result could be called genetic synergism, and perhaps it accounts for the rapidly decreasing efficiency of

established antibiotics in the treatment of some bacterial infections. The increased probability that newly discovered antibiotics will be related by cross resistance to established drugs enhances the seriousness of the problem.

Why is cross resistance important in a consideration of the application of multiple chemotherapy? An obvious reason is that the cross resistance patterns serve to divide antibiotics into a limited number of groups, all the agents within each group being relatively ineffective against bacteria resistant to any one member. Generalizations on cross resistance must always be qualified by the fact that different bacterial species, and even different strains, may show considerable individuality in over-all cross resistance pattern. Nevertheless, in most instances it would be exceptional to find bacteria of any species that were comparatively sensitive to oxytetracycline (terramycin) but resistant to chlortetracycline (aureomycin®), or vice versa. Many other cross resistances seem of general if not universal validity. Also, it is observed that cross resistance is exhibited more readily by gram-negative bacteria.

The histograms shown in Figure 2 give some limited examples of cross resistance as revealed by studies previously cited.[77,79,82,84] The figure shows cross resistance of representative bacteria. Testing was by the gradient-plate procedure, using strains selected for resistance to a single antibiotic within the group. The usefulness of drugs within each group represented in the figure is minimized by their inter-relation. Insofar as resistance is concerned, the use of one drug in a group will reduce, on the average, the potential value of all other drugs in the same "family" when employed against the same organism. However, another strain or species may respond somewhat differently. Wide departures from reciprocity in cross resistance may be encountered, suggesting for example that penicillin followed by chloramphenicol may be a more desirable sequence than administration in reverse if the former drug is to be used at high concentrations in treating gram-negative infections. Knowledge of cross resistance has induced attempts to combine unrelated antibiotics that do not affect cellular metabolism in the same manner. Even here there may be difficulty. What will occur if some relatively non-specific physiologic mechanisms exist *a priori*, or arise to produce a minor degree of decreased sensitivity to a wide variety of antibiotics? For example, a strain of M. pyogenes resistant to penicillin was found to be slightly resistant to an entire group of antibiotics related to one another by cross resistance (neomycin, streptothricin, viomycin, streptomycin and thiolutin®).[77] Penicillin has not been included in this cross resistance

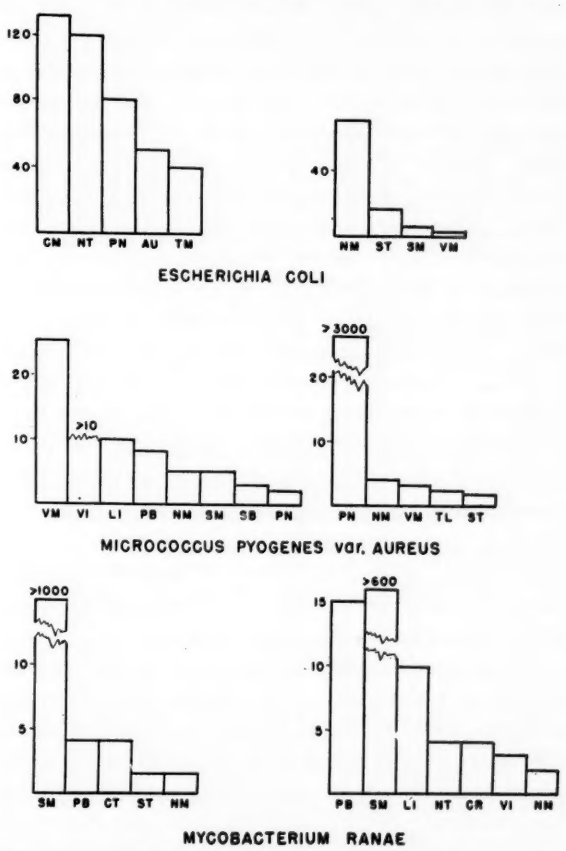

FIG. 2. Examples of cross resistance. Graphs represent the degree of increased resistance to several antibacterial agents, produced by selection in the presence of one only. Values on the ordinate represent factor of increased resistance in comparison with the parent sensitive strain. Strains were selected by exposure to the agent at the left in each group. Abbreviations: CM, chloramphenicol; NT, netropsin; PN, penicillin; AU, chlortetracycline; TM, oxytetracycline; NM, neomycin; ST, streptothricin; SM, streptomycin; VM, viomycin; VI, vinactin; LI, licheniformin-B; PB, polymyxin-B; SB, subtilin; TL, thiolutin R; CT, catenulin, and CR, circulin.

category because the cross resistance relationships between penicillin and this group are sometimes paradoxically unidirectional or of low magnitude (1.5- to 4-fold increase over the wild type). Similarly, a polymyxin-resistant E. coli is significantly more resistant than the wild type to streptothricin, neomycin, catenulin and streptomycin (3- to 10-fold), with slightly increased resistance to the unrelated netropsin®;[82] yet

polymyxin is in a separate group. These incidental relationships might ultimately be significant in the development of multiple resistance, particularly if it occurs through exponentially interactive or non-specific mechanisms.

In addition, cells that have developed consecutive resistance to several drugs have perhaps evolved a variety of different defenses, thus improving their chances of successfully resisting the activity of future antibiotics. Staphylococci are already encountered that are resistant, at least singly, to penicillin, streptomycin, the tetracyclines, erythromycin and chloramphenicol. The erythromycin-resistant strains are probably also resistant to carbomycin.[3] Cross resistance, non-specific defense mechanisms[35,41] and the consecutive development of resistance may place combination therapy in the same position within a few years that is presently occupied by drugs previously used alone. Epidemiologic studies of cross infection based on antibiotic resistance show all too clearly that many human pathogens have now had a prolonged evolutionary history of contact with antibiotics. Whether or not a human host has been exposed previously to these same drugs is becoming relatively insignificant.

We may conclude that attempts to prevent the origin of resistance by multiple chemotherapy will be only partially effective, particularly if resistance develops readily to individual components of the drug combination. The method nevertheless presents advantages over the single use of specific drugs (e.g., streptomycin or isoniazid) associated with the facile appearance of "one-step" resistant mutants.

SUMMARY

The development of drug resistance is apparently only one aspect of the continuing process of microbial evolution. Prevalence of antibiotics has resulted in the selection of new types of organisms by the principle of survival of the fittest. The changes leading to antibiotic resistance are not for the most part drug-induced but probably result from spontaneously occurring mutations leading to modified biochemical processes in the bacterial cell (resistant strains). Some microorganisms (resistant species and "naturally" resistant strains) may originally possess cytochemical systems that are not vulnerable to specific antibiotics. Categories of resistance and sensitivity are relative.

Analyses of resistance patterns show two important types, the penicillin or obligatory multistep pattern and the streptomycin or facultative one-step pattern. The type of pattern is significant in predicting the probability that resistance will develop rapidly.

Aside from the indirect evidence resulting from demonstrations of resistant clones in bacterial cultures prior to drug contact, a genetic basis for antibiotic resistance is suggested by experiments on bacterial transformation and by recombination following matings of unlike strains.

Mutations to antibiotic resistance usually include a wide variety of types, separable into different classes if sufficient criteria of examination are employed. Some disparity of results with strains of separate origin is therefore inevitable, and isolated experiments may have little statistical validity.

Gain or loss of resistance in bacterial populations, once mutations to resistance have occurred, depends primarily on selection. Here the presence or absence of antibiotics plays the major but not exclusive role.

Multiple chemotherapy is at present the most efficient method for preventing the establishment of resistant strains but it has certain potential limitations, many of which also apply to the use of drugs singly. These limitations result from the growth of resistant clones before the onset of antibiotic therapy, the possible occurrence of induced multiple mutations, the multigenic basis of some types of resistance which leads to increased probability of mutation, the increased significance of cross resistance as new antibiotics are discovered and the possibility that non-specific resistance will protect bacteria to a slight degree against several types of antibacterial agents used simultaneously allowing multiplication, mutation and selection of more highly resistant forms. Through no intrinsic fault, multiple chemotherapy must often cope with bacterial populations that have previously been allowed to develop consecutive resistances by the unsuccessful application of several antibiotics in sequence or in ineffective combination.

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polymyxin is in a separate group. These incidental relationships might ultimately be significant in the development of multiple resistance, particularly if it occurs through exponentially interactive or non-specific mechanisms.

In addition, cells that have developed consecutive resistance to several drugs have perhaps evolved a variety of different defenses, thus improving their chances of successfully resisting the activity of future antibiotics. Staphylococci are already encountered that are resistant, at least singly, to penicillin, streptomycin, the tetracyclines, erythromycin and chloramphenicol. The erythromycin-resistant strains are probably also resistant to carbomycin.[3] Cross resistance, non-specific defense mechanisms[35,41] and the consecutive development of resistance may place combination therapy in the same position within a few years that is presently occupied by drugs previously used alone. Epidemiologic studies of cross infection based on antibiotic resistance show all too clearly that many human pathogens have now had a prolonged evolutionary history of contact with antibiotics. Whether or not a human host has been exposed previously to these same drugs is becoming relatively insignificant.

We may conclude that attempts to prevent the origin of resistance by multiple chemotherapy will be only partially effective, particularly if resistance develops readily to individual components of the drug combination. The method nevertheless presents advantages over the single use of specific drugs (e.g., streptomycin or isoniazid) associated with the facile appearance of "one-step" resistant mutants.

SUMMARY

The development of drug resistance is apparently only one aspect of the continuing process of microbial evolution. Prevalence of antibiotics has resulted in the selection of new types of organisms by the principle of survival of the fittest. The changes leading to antibiotic resistance are not for the most part drug-induced but probably result from spontaneously occurring mutations leading to modified biochemical processes in the bacterial cell (resistant strains). Some microorganisms (resistant species and "naturally" resistant strains) may originally possess cytochemical systems that are not vulnerable to specific antibiotics. Categories of resistance and sensitivity are relative.

Analyses of resistance patterns show two important types, the penicillin or obligatory multistep pattern and the streptomycin or facultative one-step pattern. The type of pattern is significant in predicting the probability that resistance will develop rapidly.

Aside from the indirect evidence resulting from demonstrations of resistant clones in bacterial cultures prior to drug contact, a genetic basis for antibiotic resistance is suggested by experiments on bacterial transformation and by recombination following matings of unlike strains.

Mutations to antibiotic resistance usually include a wide variety of types, separable into different classes if sufficient criteria of examination are employed. Some disparity of results with strains of separate origin is therefore inevitable, and isolated experiments may have little statistical validity.

Gain or loss of resistance in bacterial populations, once mutations to resistance have occurred, depends primarily on selection. Here the presence or absence of antibiotics plays the major but not exclusive role.

Multiple chemotherapy is at present the most efficient method for preventing the establishment of resistant strains but it has certain potential limitations, many of which also apply to the use of drugs singly. These limitations result from the growth of resistant clones before the onset of antibiotic therapy, the possible occurrence of induced multiple mutations, the multigenic basis of some types of resistance which leads to increased probability of mutation, the increased significance of cross resistance as new antibiotics are discovered and the possibility that non-specific resistance will protect bacteria to a slight degree against several types of antibacterial agents used simultaneously allowing multiplication, mutation and selection of more highly resistant forms. Through no intrinsic fault, multiple chemotherapy must often cope with bacterial populations that have previously been allowed to develop consecutive resistances by the unsuccessful application of several antibiotics in sequence or in ineffective combination.

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Present Status of the Chemotherapy of Tuberculosis*

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THE spectacular advances in the therapy of tuberculosis in the past decade together with enthusiastic press reports and the closing of a few sanitoria have led to the widespread belief that tuberculosis is no longer a problem. It should be remembered that the decline in tuberculosis mortality antedated the introduction of modern chemotherapy by a good many years, and it is instructive to remember that predictions were being made about the imminent disappearance of tuberculosis at the turn of the century.[1]

There are certain facts about tuberculosis which should temper our enthusiasm for the triumphs of chemotherapy. Tuberculosis is characteristically a chronic relapsing disease. It is sobering to realize that in the days before chemotherapy a person with active minimal pulmonary tuberculosis had a 20 per cent chance of being dead, or having active disease, ten years after the diagnosis was made,[2] and a patient diagnosed as having far advanced pulmonary disease had a 70 per cent chance of death or active disease ten years later.[3] Certainly the odds have improved greatly but it must be remembered that short-term results of chemotherapy have a limited meaning and a careful study of relapse rates is essential for final evaluation of the modern therapy of tuberculosis.[4,5]

RELATIONSHIP OF PATHOLOGY OF TUBERCULOSIS TO CHEMOTHERAPY

Before considering the problems of chemotherapy of tuberculosis it is helpful to review some of the aspects of its special pathology and bacteriology for these reveal the reasons for its chronic and relapsing nature.

The initial reaction of human tissues to the tubercle bacillus must be inferred from studies in experimental animals, since human infection has never been recognized in the prenecrotic stage even in autopsies of persons suffering sudden death.[6] Koch[7] described the phenomenon in the experimental animal as follows, "If a normal guinea pig is inoculated with a pure culture of tubercle bacilli, the wound, as a rule, closes and in the first few days seemingly heals. After ten to fourteen days, however, there appears a firm nodule which soon opens, forming an ulcer that persists until the animal dies." This classic description emphasizes two important aspects of the disease. The initial reaction of tissue of the tubercle bacillus is minimal, and during this period there may be widespread dissemination of bacilli. If one observes *in vivo* the initial response to tuberculous infection in the rabbit ear chamber,[8] one sees the gradual accumulation of exudate around the inoculum. Coincident with the development of hypersensitivity, there is a dramatic change in the character of the reaction. There is a much more violent inflammatory response characterized by further outpouring of exudate together with stasis and thrombosis of small blood vessels, resulting in necrosis of the involved area. It is the development of allergy to tuberculoprotein which results in the characteristic destructive nature of tuberculous infection, and the necrotic tubercle which is recognized as the most important unit lesion of tuberculosis results from this hypersensitive reaction to the tubercle bacillus.[9]

Tuberculous infection in this country is almost always airborne, which means that pulmonary tuberculosis is the most common and most important type of tuberculosis. For this reason it is pertinent to consider some of the particular aspects of pulmonary pathology. Pulmonary infection always begins as a pneumonitis,[10] which means that the involved area communicates with a bronchus. Exudate may persist for a long time and all areas of exudate do not become necrotic. However, necrosis always

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occurs in some part of the infected area. The necrotic lesion in the lung may be a "closed lesion" which is solid and retains the necrotic stroma of the normal lung, or the necrotic tubercle may liquefy and either spill its contents into a communicating bronchus to produce a cavity, or retain the necrotic debris and become a "closed cavity." Medlar,[10] by careful dissection of necrotic pulmonary lesions, has demonstrated that usually the bronchus to a necrotic focus in the lung is patulous even though plugged with necrotic debris. He has emphasized the importance of this type of lesion. "It is the open bronchial communication with the apparently walled off necrotic pneumonic lesion together with the potential, although unpredictable, liquefaction of the area that causes pulmonary tuberculosis to be characteristically a chronic relapsing disease."

Within the caseous tubercle and the cavity, and even within mononuclear cells, tubercle bacilli may survive for long periods.[1,11] In the aerobic atmosphere of the cavity they not only survive but also actively multiply, and the spread of infection to other parts of the lung is a constant threat. Tubercle bacilli in closed caseous lesions are metabolically less active and are confined momentarily, but liquefaction of the necrotic area may at any time allow the liberation of these bacilli to other parts of the lung.

In summary, tuberculosis is a disease characterized by tissue destruction. Tubercle bacilli may exist in a variety of metabolic states both intra- and extracellularly and the bacilli may actively multiply or they may remain dormant for months or years. To be completely effective against this infection a drug must be able to penetrate caseous areas and cell membranes, it must be able to act in a variety of environments, and it must be able to influence growing and dormant bacilli.

RECENT HISTORY OF CHEMOTHERAPY

The present status of chemotherapy of tuberculosis cannot be fully appreciated apart from its historical development since the basic principles of chemotherapy today have evolved from the studies of the past. This section will be divided into topics which represent different phases in the evolution of drug treatment.

Pre-streptomycin Period. The modern chemotherapy of tuberculosis can be said to have begun with the discovery in 1938 that sulfanilamide had a limited suppressive effect on experimental tuberculosis in guinea pigs.[12] Studies with sulfonamide compounds led to the introduction of the sulfone compound promin and the demonstration by Feldman and his co-workers[13] that this drug had a definite therapeutic effect on experimental tuberculosis in guinea pigs. Clinical trials were attempted with promin and there was evidence that it had some effectiveness, but the medical profession was skeptical and Feldman[14] relates how coldly these clinical results were received when first presented. There was a widespread belief at this time that effective drug treatment of tuberculosis was an impossible goal and only a small group of investigators retained their faith that more effective drugs could be developed. The general attitude of the medical profession changed abruptly with the introduction of streptomycin.

Early Streptomycin Period. Toxicity and resistance: In 1944 Shatz, Bugie and Waksman[15] published their first report on streptomycin (SM) and noted its antibiotic activity against gram-positive and gram-negative bacteria. A short time later[16] they reported the effect of streptomycin upon mycobacterium tuberculosis and noted that it was highly effective *in vitro.*

Knowing the interest of the Mayo group in the chemotherapy of tuberculosis, Dr. Waksman asked Dr. Feldman and Dr. Hinshaw to test streptomycin *in vitro.*[14] The amount of drug available for testing was small and only thirty guinea pigs were used in the initial animal experiment but the results were so striking[17] that clinical trial was planned immediately. Thirty-four patients were treated by Hinshaw and Feldman beginning in December, 1944, and this clinical study substantiated the animal experiments.[18] Streptomycin had an unequivocal effect on tuberculous infection in man. It was reported, however, that deafness and vestibular dysfunction occurred in some cases and exacerbation of the disease was noted occasionally after the drug was stopped. Thus the earliest clinical trial of this antibiotic established its most important toxic signs and suggested that the drug suppressed tuberculous infection but did not eradicate it.

Subsequent clinical trials amply confirmed these conclusions and established that the other limiting factor in therapy was the development of streptomycin resistance. In 1946 Youmans et al.[19] reported that the tubercle bacilli recovered in cultures from eight of twelve patients re-

ceiving streptomycin were resistant to 500 to 1,000 times the amount of streptomycin initially sufficient to inhibit the bacilli. They also noted that streptomycin resistance could be produced *in vitro* by serial passage of tubercle bacilli through media containing increasing increments of streptomycin.

In January, 1946, the Committee on Chemotherapy and other agencies of the National Research Council began a small but intensive study of the toxicity of streptomycin under the direction of McDermott.[20] This investigation substantiated the frequent occurrence of eighth nerve damage, as evidenced by vestibular dysfunction and, less frequently, deafness, as the major toxic effect of streptomycin. Anaphylactic reactions, eosinophilia and cylinduria were also described. Subsequent reports[21,22] noted leukopenia and rarely agranulocytosis, drug fever and rash, circumoral paresthesia, and rarely jaundice as other toxic effects.

It became evident in 1945 that streptomycin was the most effective drug which had ever been developed against tuberculous infection but that adequate study of its clinical effectiveness would require large cooperative studies. This was a new field in terms of tuberculosis research and there were few precedents to guide the investigators who set up the study program. Much of the data which evolved from these early studies was inadequate but much was learned and standardized methods for clinical evaluation were rapidly developed.

Various groups cooperated to evaluate this new drug. In 1946 and 1947 the American Trudeau Society and the U. S. Public Health Service set the framework for a cooperative study on various aspects of streptomycin therapy. The Public Health Service appointed a tuberculosis Study Section for this purpose.[23] Parts of this study were reported in the book edited by Riggins and Hinshaw published in 1949.[24]

In May, 1946, the Army, Navy and Veterans Administration concluded that sufficient data had accumulated on the efficacy of streptomycin to justify an investigation into the effect of streptomycin upon pulmonary tuberculosis in man. A plan of study was set up by these three federal agencies with the help of the U. S. Public Health Service, the National Research Council and the National Tuberculosis Association.[25] This was the beginning of one of the most productive studies on the clinical management of a single disease which has ever been designed. It has continued until the present and its annual chemotherapy conferences have had tremendous influence on the current management of tuberculous infection.

In September, 1946, the British Medical Research Council began to organize its first clinical trial of streptomycin.[26] In many ways this trial and those which followed have been the most carefully planned cooperative studies and have given the most comparable information from one trial to the next. The British trials are limited, however, in the number of cases treated and the types of tuberculosis studied.

Because the initial chemotherapy trial of the M.R.C.[26] was so carefully planned, and because it alone contained a group of untreated controls, it is worth while considering in more detail its conclusions for they are basically the same conclusions that all the preliminary cooperative studies achieved. In setting up the study the planning board decided to treat only acute progressive bilateral pulmonary tuberculosis of presumably recent origin, bacteriologically positive, unsuitable for collapse therapy and in the age group of fifteen to thirty years. This type of disease was chosen for several reasons. It was believed that a suitable control group could be studied since bedrest alone would be the ordinary treatment provided for such patients. It would provide a relatively uniform type of disease which ordinarily had a high mortality and, by limiting the age groups studied, factors other than the stage of the disease would not have much influence on treatment. Patients were selected as suitable for trial by a central committee and were distributed between the streptomycin-treated group and the control (bedrest) group by random sampling. Fifty-two cases served as controls, and fifty-five cases were treated with streptomycin 2 gm. daily in four divided doses. The groups were therefore as comparable as it was possible to make them.

Treatment was given daily for four months and cases were evaluated at six months and again at twelve months. Clinical improvement in terms of weight gain, change in sedimentation rate and fall in fever was greater in the streptomycin-treated group than in the controls but these changes were not as striking as the radiologic changes. Table 1 summarizes the results at the end of six months. Bacteriologically, the results were not impressive, and a high incidence of bacterial resistance to streptomycin was found. Clinical deterioration was closely related

to the development of bacterial resistance. Toxic side effects were common and 65 per cent of the streptomycin-treated patients had some degree of vestibular dysfunction.

At the end of twelve months' observation the difference between the two groups, in terms of improvement, was not as striking as at six months. Fifty-six per cent of the treated group showed persistent improvement and 31 per cent of the controls. There had been twelve deaths (22 per cent) in the treated group as compared with twenty-four (46 per cent) in the control group.

TABLE I
STREPTOMYCIN TREATMENT COMPARED WITH UNTREATED CONTROLS*

	X-ray Improvement, All Grades (%)	X-ray Deterioration (%)	Deaths (%)	Negative Sputum on Culture (%)	SM-Resistant Bacilli (as % of positives)
Controls—bed-rest (52 cases)	33	34	27	4	..
SM-treated (55 cases)	69	20	7	14	85

* Patients in SM-treated group were given SM 2 gm. daily for four months. These are results for two months after stopping therapy. Material taken from British Medical Research Council Report.[26]

The inevitable conclusions were that streptomycin therapy exerted a profound effect on the clinical course of pulmonary tuberculosis but that chemotherapy was far from being definitive therapy; and the limiting factors of treatment were the development of streptomycin resistance and toxicity. The Army-Navy-Veterans Administration study[25,27,28] and the American Trudeau Society study[24] all came to approximately the same conclusions. During this period it was thought that chemotherapy should be reserved for cases in which there was predominantly exudative disease, and should not be used in disease which could be expected to do well on traditional management.

The problems which required exploration were: (1) did SM resistance *in vivo* mean the cessation of therapeutic effectiveness, (2) could the development of resistance be delayed by altering dosage, and (3) could therapeutic effectiveness be maintained at the same time that toxicity was reduced by changing the dosage?

Ample evidence was presented experimentally that SM-resistant tubercle bacilli were virulent[29–31] and experimental infection with those organisms was unaffected by treatment with streptomycin. Steenken[31] found that in experimental infection resistance to 10–15 μg. was the critical level. At levels of resistance below 10 μg. good response to therapy could be obtained and above 15 μg. no response could be expected.

TABLE II
COMPARISON OF THERAPEUTIC EFFICACY AND TOXICITY OF VARIOUS STREPTOMYCIN REGIMENS*

Dosage of Streptomycin	Therapeutic Efficacy of Cases Showing Improvement (%)	Patients Developing Toxic Symptoms (%)
1.8–2.0 gm. daily, 120 days	80	80
2 gm. daily, 60 days	72	63
1 gm. daily, 120 days	83	27
0.5 gm. daily, 120 days	83	7

* Results of Veterans Administration, Army, Navy Cooperative Study.[22]

The Veterans Administration, Army and Navy cooperated in a study of various regimens of SM and in a report on 2,780 patients treated at forty-eight hospitals[22] it was noted that reduction of dosage from 2 gm. daily to 1 gm. daily for 120 days did not reduce the therapeutic effectiveness of the drug and caused moderate reduction in toxicity, and that 0.5 gm. daily was as effective and was accompanied by a marked reduction in toxicity from 80 per cent to 7 per cent. (Table II.) It was found that after four months treatment approximately 50 per cent of patients still had positive cultures, and 70 per cent of cultures still positive were SM-resistant. Attempts to shorten the period of therapy to forty-two days in an effort to solve the problem of resistance[32] were not successful in terms of maximum therapeutic effect. In 1948 dihydrostreptomycin[33,34] was introduced with the hope that toxicity could be reduced but it was soon discovered that streptomycin produced more vestibular damage and dihydrostreptomycin caused eighth nerve deafness so that the problem of toxicity was not solved.[35,36] The drugs were found to be identical in their chemotherapeutic effect and bacterial resistance to one meant resistance to the other.

The major advance during this period was the

reduction in dosage of SM, without significant loss of therapeutic effect but with considerable reduction in toxicity.

Combined Chemotherapy. Long-term treatment: In 1946 Lehmann[37] described the antituberculous effect of *para-aminosalicylic acid* (PAS) and subsequent studies demonstrated that this drug had a definite although limited effect on clinical tuberculosis. It had the major disadvantage that it had to be given in large doses and gastrointestinal symptoms were troublesome.[38] By 1948 the limitations of chemotherapy with streptomycin alone were obvious and it was natural to search for some method of prolonging bacterial sensitivity to SM. In 1948 the Veterans Administration[36] began a pilot study with combined SM and PAS and the results were promising enough to warrant a full-scale trial of combined therapy in 1949. Laboratory studies and early clinical trials indicated that SM and PAS were more effective *in vivo* and *in vitro* than either drug alone and suggested that more prolonged therapy might be feasible using these drugs in combination.[39–41]

TABLE III
COMPARISON OF PAS ALONE AND IN COMBINATION WITH SM*

Dosage	X-ray Improvement Considerable and Moderate (%)	Sputum Conversion on Culture (%)	Streptomycin Resistance. Moderate and Strongly Resistant (as % of positive cultures)
PAS alone, 20 gm./day (59 cases)	54	7	..
SM alone, 1 or 2 gm./day (109 cases)	71	16	76
SM 1 gm./day and PAS 5 or 10 gm./day (73 cases)	88	25	34
SM 1 gm./day and PAS 20 gm./day	87	23	6

* Observations of six months after start of treatment. Treatment given for three months. Results of British Medical Research Council.[42]

A study of combined chemotherapy was undertaken by the M.R.C. about the same time and was reported in 1950.[38] This study was set up in a manner similar to the first M.R.C. trial of streptomycin except that no untreated control group was included. Three groups were compared, PAS alone in the dosage of 20 gm. daily, SM alone, 1 gm. daily, and PAS 20 gm. plus SM 1 gm. daily. Treatment was given for three months and observations were made at three months and at six months. It was found that PAS alone had a definite beneficial effect as compared with the original control group in the first M.R.C. trial[26] but that this effect was appreciably less than that of SM alone. Although combined SM and PAS therapy was slightly superior to SM alone in terms of sputum conversion and x-ray improvement, the differences were not great. The striking difference was in the emergence of drug-resistant organisms. Streptomycin-resistant strains were isolated in thirty-three of forty-nine streptomycin-treated cases and in only five of forty-eight cases treated with both SM and PAS.[38,42] (Table III.)

Clinical relapse was more likely to occur in patients in whom SM resistance developed but it was noted that even patients with SM-resistant organisms sometimes continued to improve. It was pointed out that the entire bacterial population was not resistant even though resistant organisms were isolated in the sputum.

The Veterans Administration-Army-Navy group selected three basic regimens for study:[36] (1) SM 1.0 gm., daily plus PAS 12.0 gm. daily; (2) SM 0.5 gm. daily, plus PAS 12.0 gm. daily; and (3) SM 1.0 gm. twice a week, plus PAS 12.0 gm. daily. It was determined that the chemotherapeutic effect of these regimens was the same in patients treated for the first time ("original treatment care") but that toxicity and development of streptomycin resistance was lowest on the third regimen, 12 gm. of PAS plus 1 gm. SM twice a week. Patients who had been treated before with SM ("retreatment cases") did not respond as well to combined therapy even though the tubercle bacilli were still sensitive.[43]

Perhaps the most important discovery made in this period was that long-term treatment was superior to therapy for three to four months.[43–45] Actually, the results with combined therapy after four months therapy were little better than with SM alone. It was found, however, that continued chemotherapy on any of the basic regimens resulted in a steady improvement in

terms of x-ray changes and sputum conversion, so that instead of 40 to 50 per cent moderate to marked x-ray improvement at four months there was 70 per cent (Table IV) after twelve months.

TABLE IV
EFFECT OF DURATION OF TREATMENT WITH SM + PAS ON THERAPEUTIC RESULTS*

Dosage	X-ray Improvement Moderate and Marked (%)	Sputum or Gastric Conversion of Culture (%)
SM + PAS 3 to 4 mo.	57	50
SM + PAS 9 to 12 mo.	75	70

* Evaluation at twelve months after start of therapy. Results of Veterans Administration: Army, Navy Study (Tucker, W. M.[43]).

As might be expected, x-ray improvement and sputum conversion were achieved at higher rates in moderately advanced cases than in far advanced tuberculosis and resistance developed more often in patients with large cavities.[43]

The advances made in this period were the reduction in the incidence of drug resistance by the use of combined drug therapy, and the concept of long-term treatment. The efficacy of PAS-SM therapy with either daily SM or intermittent SM gave a valuable yardstick for the evaluation of new drugs.

Isoniazid. In 1952 isoniazid (isonicotonic acid hydrazide) and its isopropyl derivatives were introduced as chemotherapeutic agents for the treatment of tuberculosis. The preliminary reports from Sea View Hospital[46] and the New York Hospital[47] indicated that these compounds were highly potent agents and extensive studies were begun immediately. Rapid clinical evaluation of isoniazid (INH) was possible since the framework of large cooperative studies was now firmly established. It soon became apparent that the isopropyl derivative of isoniazid was more toxic and no more effective than isoniazid[48] so that it has not been used in any of the large cooperative studies, and is not now in general clinical use.

The studies of the V. A.-Army-Navy group,[36] U. S. Public Health Service[49,50] and Medical Research Council of Great Britain[51,52] all arrived at approximately the same conclusions in evaluating this new drug alone and in combination with SM or PAS. It was found that isoniazid alone was not as effective as isoniazid plus PAS or INH plus SM in terms of x-ray improvement or sputum conversion, and isoniazid resistance developed much more rapidly when INH was given alone. (Table V and VI.)

TABLE V
COMPARISON OF VARIOUS REGIMENS WITH AND WITHOUT INH*

Drug Regimen	X-ray Improvement, All Grades (%)	Sputum Conversion on Culture (%)	INH Resistance (as % of positives)
SM + PAS	64	55	.....
INH	54	37	62 (35% of total cases)
SM + INH	64	67	13 (3% of total cases)

* Results after three months of treatment. Taken from British Medical Research Council.[51]

TABLE VI
COMPARISON OF INH + SM AND INH + PAS*

Drug Regimen	X-ray Improvement, All Grades (%)	Sputum Conversion on Culture (%)	INH Resistance (as % of positives)
SM 1.0 gm./day plus INH 200 mg./day	82	65	5
PAS 20 gm./day plus INH 200 mg./day	76	66	0

* Results after three months treatment. British Medical Research Council Trials.[52]

Although isoniazid alone did not seem to be as effective as isoniazid combined with SM or PAS, it did seem to be a remarkably potent drug when given alone and the number of relapses which occurred after cessation of therapy did not seem as great as when SM was given alone. In the routine laboratory analysis of this drug a

surprising fact was brought to light.[53–55] It was found that isoniazid-resistant organisms were often (though not invariably) avirulent in the guinea pig and there was natural speculation about its virulence in other animals and in man. The virulence of isoniazid-resistant organisms for mice was soon established[56] and the clinical observation was made repeatedly that clinical deterioration may occur in patients with isoniazid-resistant organisms[51,57] so that this simple solution to the treatment of tuberculosis did not prove to be a fact. Nevertheless, the discovery that isoniazid-resistant organisms are altered in their virulence for at least one species, and the finding that they have different growth requirements and different biochemical reactions[58,59] compared with INH-sensitive organisms gives rise to the speculation that their virulence in the human may be altered if not nullified. McDermott and his co-workers[60] have indicated that in their hands isoniazid alone is a remarkedly effective drug and emphasize that from the public health viewpoint it may prove a valuable weapon when used alone in the treatment of the ambulatory patient. Isoniazid has the great advantage that it can be given orally and in relatively low dosage.

Although a wide variety of toxic effects of isoniazid have been recorded, toxicity has not been a major problem with the doses usually given. Most of the toxic effects of the drug are related to the central nervous system.[46,47,49] Hyperreflexia is common, and peripheral neuritis, toxic psychoses and acute convulsive reactions may occur. Pyridoxine deficiency has been reported with high doses of INH[61] and it was claimed that peripheral neuritis can be prevented by giving pyridoxine (50–450 mg./day). Vitamin B_{12} also has been reported to be effective in treatment of peripheral neuritis occurring in patients with this complication of INH therapy;[62] allergic reactions including chills, fever, dermatitis, arthralgia and purpura have been recorded, as have hemolytic anemias.[63]

In the early reports of this drug it was noted that it had a specific euphoric effect. It is likely that this was more closely related to the enthusiasm of the investigators than to the specific effect of the drug since careful studies have failed to substantiate this finding.[64,65] Withdrawal symptoms consisting of nightmares, head consciousness, nervousness and depression have been noted after sudden cessation of therapy, and it is usually recommended that the drug be stopped by gradual lowering of the dosage.[66]

One other remarkable fact has emerged from clinical studies with isoniazid which indicate that it is a particularly potent drug. Miliary tuberculosis or tuberculous meningitis have not been observed to develop during therapy with INH whereas these complications have been known to arise in patients treated with SM-PAS.[67] In a recent study of acute miliary tuberculosis it was found that eight of twenty-four patients treated with SM and PAS developed meningitis during therapy but none of twelve patients treated with INH alone or in combination developed this complication.[68]

The introduction of isoniazid provided the most potent single drug for the treatment of tuberculosis that has been developed to date. More important, it gave the physician a choice of regimens. Before the introduction of INH, SM plus PAS was the treatment of choice and the only really effective treatment. This could now be supplemented with INH plus PAS, INH plus SM, and even INH alone.

Minor Drugs. A tremendous number of drugs have been screened *in vitro* for their antituberculous effect, fewer have had some *in vivo* trial in animals, and a number of these have had clinical trial. A few of these are worthy of mention for they have certain usefulness in particular cases. Viomycin[70–74] is approved and available for general use. Toxicity and rapid emergence of resistance make it useful only in situations in which neither streptomycin nor isoniazid can be used. Pyrazinamide[75–77] is not a very potent drug alone but combined with INH seems highly effective.[76,78] It is of considerable theoretic interest because experimentally it has been possible to eradicate infection in mice with pyrazinamide plus INH.[78,79] A high degree of hepatotoxicity makes its prolonged use impractical.[78] Oxytetracylene[80,81] has been substituted for PAS effectively for use with streptomycin but clinical trials with this drug are not extensive and its ultimate value is in doubt.

IDEAL CHEMOTHERAPEUTIC AGENT

Much has been accomplished in the treatment of tuberculosis during the past decade. A number of useful drugs are available and much has been learned about their administration. However, the problem of chemotherapy of tuberculosis has not been solved.

Before reviewing the specific merits and

defects of the drugs now in general use it might be well to postulate the properties of an "ideal drug" for in this way we can understand better the problems which remain. To be effective, the "ideal drug" should be bactericidal or bacteriostatic in relatively low concentrations. It should be able to accomplish this without the development of bacterial resistance. The drug must be highly diffusible and be able to reach all tissues including the meninges and brain. And it should be able to penetrate avascular necrotic foci in sufficiently high concentrations to have an effect on the tubercle bacillus. Since tubercle bacilli may survive and multiply within mononuclear cells, the "ideal drug" must be able to penetrate the cell membrane. Mere availability of the drug is not enough, however, for it must be effective in a variety of biochemical environments,[82] particularly that which prevails within the caseous or necrotic lesion. It must also be effective against organisms which exist in a variety of metabolic states ranging from organisms actively dividing to those which appear dormant. It must be relatively non-toxic in therapeutic doses and toxicity must remain low even when the drug is given for protracted periods. Finally, it must be relatively stable for reasonable lengths of time, and not excreted so rapidly or altered so quickly by the host that it is impossible to obtain therapeutic levels.

Obviously such a "perfect drug" would be capable of eradicating tuberculous infection. McDermott[82] has emphasized that the distinction between bactericidal and bacteriostatic drugs is an artificial one since a drug bactericidal for actively dividing bacilli may not affect metabolically dormant organisms and a bacteriostatic drug may possess the potential for eradicating infection if bacteriostasis is sufficiently prolonged. It was noted earlier that tuberculosis is characteristically a chronic relapsing disease because tubercle bacilli may survive for long periods in caseous lesions. Obviously, the only absolute safeguard against relapse is eradication of the infection.[78,82]

How far short of the ideal are we today? A review of what is known about the drugs in use today in terms of the "ideal drug" will help to clarify this question.

Bactericidal or Bacteriostatic in Low Concentrations. Streptomycin and isoniazid are both potent bacteriostatic drugs in low concentrations. Streptomycin is effective against most human strains of mycobacterium in concentration of 0.2–1.0 μg./ml. *in vitro* using Dubos tween-albumin media.[83] Isoniazid will produce inhibition of growth in concentrations as low as 0.05 μg./ml.[84] PAS produces inhibition of growth at 1.2 μg./ml. but inhibition is never complete.[39] The bacteriostatic effect of PAS *in vitro* is closely related to the size of the inoculum and if the inoculum is increased tenfold much higher concentrations of PAS are necessary to produce inhibition.[85] The problem of whether or not INH and SM are bacteriostatic or bactericidal drugs under certain conditions *in vitro* is not germane to the present discussion. Whatever their action *in vitro*, they will not eradicate infection *in vitro*.[79,86] In summary, streptomycin and isoniazid are highly effective *in vitro* at a level easily attained *in vivo*. PAS is less effective than either of these drugs.

Development of Bacterial Resistance. All of the drugs in routine use have the common failing that bacterial resistance develops readily both *in vitro* and *in vivo*, and in this sense they fall short of the ideal. In the British M.R.C. trials 70 per cent of positive cultures were resistant to significant levels of SM after three months treatment with that drug alone.[26] After three months treatment with PAS alone 24 per cent of cultures remaining positive were resistant[42] and after three months of therapy with isoniazid 64 per cent of cultures still positive were resistant.[83] Resistance to PAS develops more slowly but nonetheless it does occur. The mechanism of bacterial resistance is not within the scope of this paper and will not be discussed. It is important to note, however, that mixed populations of resistant organisms may occur *in vivo* and the finding of resistant organisms in the sputum may not be an absolute contraindication to combined therapy.[42]

The use of combined chemotherapy has been an important step toward solving the practical problem of bacterial resistance. All of the cooperative clinical trials have shown a delay in the development of bacterial resistance to SM and isoniazid when combined therapy is used.[36,38,42,43,52]

Theoretically, combined therapy should reduce geometrically the development of bacterial resistance, and yet a small percentage of patients continue to have positive sputum and cultures become drug resistant. Mitchison[85] discusses this problem at some length and points out certain theoretic reasons for this. To begin with, the most plausible reason is that cultures

are not sensitive to one of the drugs at the start of therapy. Since the occurrence of naturally drug-resistant mutants is rare, this is usually due to previous therapy with one of the drugs, particularly streptomycin. Mitchison postulates that during intermittent streptomycin therapy (twice weekly), isoniazid-resistant bacilli may multiply during periods when streptomycin is not given; and when these resistant organisms become numerous enough, mutants resistant to streptomycin may then develop.

Distribution of Drug In Vivo. All three of the drugs in general use seem to be absorbed readily and blood and tissue levels obtained are adequate.[47,87,88] Streptomycin has been demonstrated in cavities[89] in amounts adequate for bacteriostasis. Isoniazid labeled with C_{14}[88] has been shown to penetrate caseous areas in high concentrations, and to penetrate the dense fibrous capsule of caseous lesions. Little is known about the ability of PAS to penetrate caseous areas but it is likely that it does.

Penetration of Cell Membrane. Working independently, Suter in this country and Mackaness in England have worked out methods for culturing monocytes which have been infected with tubercle bacilli.[90,91] The methods are quantitative and allow a measure of the inhibition of growth of bacilli within the cell by various agents. Both have found[90–92] that isoniazid inhibits the multiplication of intracellular tubercle bacilli in the same concentration as it does in the test tube. In contrast, twenty times as much streptomycin is necessary to inhibit intracellular organisms than is required to inhibit bacilli grown on synthetic media. PAS has no effect on the intracellular organisms.[91]

Biochemical Environment of the Lesion. Even though a drug penetrates an avascular area this does not mean that it will be effective. The biochemical environment of the caseous lesion[82] may alter the action of a drug. For example, the lower pH in caseous areas may provide a less favorable environment for the action of streptomycin. Little is known about this but it may partially explain why a drug may be bactericidal *in vitro* but fail to eradicate infection *in vivo*.

Metabolic States of the Tubercle Bacillus. It has been observed by a number of investigators[93,94] that streptomycin and isoniazid have less effect *in vitro* against organisms which are resting than against those which are actually multiplying. The biochemical environment of the caseous lesion is unfavorable for the growth and optimum metabolism of the tubercle bacillus and this very fact may mean that the bacillus which is dormant and survives in the caseous area is less susceptible to drug therapy.[86]

Toxicity. None of the drugs now in general use is non-toxic but, by manipulation of dosage, toxicity is not a major barrier to effective therapy. The major toxicity of SM is eighth nerve damage and this can be reduced greatly by reduction of dosage.[32] Isoniazid is relatively non-toxic and the central nervous system symptoms and peripheral neuritis noted have necessitated interruption of therapy only in about 1 per cent of cases.[50] PAS is the most difficult drug to use clinically because of the high incidence of gastrointestinal symptoms which it produces. In this country it is rarely possible to give more than 12 gm. daily although the British M.R.C. trial reports the use of 20 gm. routinely.

Streptomycin should not be used in the presence of renal impairment since levels become so elevated if kidney damage is present that eighth nerve damage is likely to occur in patients with previous histories of convulsions or psychoses. In such individuals the drug should be discontinued on the first indication of a toxic effect on the central nervous system.[95]

Hypersensitivity to all three drugs occurs and should be watched for since such a reaction requires cessation of treatment. PAS is the worst offender. Occasional fatal cases have been described.[95]

Stability. Streptomycin and isoniazid are relatively stable drugs but PAS deteriorates on standing and there seems to be considerable variation in the purity of various preparations of PAS.[96]

Little is known about how these drugs may be altered *in vivo*. Hughes[97] has shown recently that in certain patients relatively little unchanged isoniazid may be present in the blood and urine, and that patients vary greatly in their capacity to acetylate the compound. Nothing is known about what happens to these drugs in caseous lesions in terms of inactivation. It may be that certain therapy failures are due to inactivation of the drug by the host.

RESIDUAL CASEOUS LESION

It is apparent that the ideal chemotherapeutic agent has not been attained and eradication of infection cannot be guaranteed. Since bacilli survive in caseous areas and these areas are the

probable source of re-exacerbation of infection, it was suggested by Medlar that residual caseous lesions be resected.[98] A number of groups followed this suggestion[99,100] and it was natural that there should be great interest in the bacteriology of these resected lesions. It was consistently found that only a small percentage (2 to 24 per cent) of such lesions yielded bacilli on culture or guinea pig inoculation in spite of the fact that bacilli could often be seen on direct smear. This led to considerable speculation about the status of such lesions; were they sterile and were the organisms really dead, or were the bacilli in a dormant stage? The implications were obvious for if the bacilli were really dead why take out residual caseous areas? At the height of this controversy Dubos[86] pointed out that it was common experience to be unable to culture bacilli from caseous lesions even without chemotherapy. He noted that substances could be found in normal tissue which inhibit the growth of tubercle bacilli. These include spermine and spermidine activated by the tissue enzyme spermine oxidase, lysozyme and a basic polypeptide isolated from the thymus. He also pointed out that organic acids accumulate around tuberculous lesions, and that tubercle bacilli are unable to multiply in ordinary media after exposure for several weeks to physiologic concentrations of certain organic acids. Conditions therefore exist in caseous lesions which are not conducive to optimum growth of tubercle bacilli. He noted at the same time that bacilli are probably not as susceptible to chemotherapeutic agents when they are metabolically inactive, and voiced caution about being certain that such bacilli were in fact dead.

Recently, Hobby et al.[101] were able to recover bacilli on culture from twenty-five of thirty-one closed lesions which had been resected. In nine of the lesions viable bacilli were isolated only after prolonged incubation, and when isolated were fully virulent for guinea pigs. Thus they demonstrated that the failure of routine cultural methods to demonstrate viable tubercle bacilli does not necessarily mean that the organisms are dead.

What is the answer to this vexing question of the resection of residual caseous foci? It seems unlikely that it will become a widely accepted principle of therapy for several reasons. First, it is probably impossible to remove all caseous foci successfully, and no one can predict which lesion will be the source of re-exacerbation of the disease. Secondly, the very fact that tubercle bacilli from caseous areas must be so tenderly nurtured in order to revive them after prolonged chemotherapy suggests that they are less capable of producing future exacerbation of the disease. It seems likely that prolonged chemotherapy augments the natural inhibition of tubercle bacilli by the environment of the caseous focus, even though drugs are less effective against the metabolically inactive bacillus than the bacillus which is growing.

PRACTICAL CONSIDERATIONS

Who Should Be Treated. With the introduction of new drugs and the accumulation of experience in the use of these agents in the past decade there has been a gradual change in attitude concerning what forms of disease should be treated. There was never any doubt that meningitis, miliary disease and the acute exudative forms of pulmonary tuberculosis should receive therapy. Indeed, it has been stated that chemotherapy is probably effective only in the more acute forms[25] but gradually, as improvement was noted in more chronic types of disease, it has become the general view that all types and all stages of the disease are benefited by chemotherapy. Today, it is generally agreed that any form of active tuberculosis should receive chemotherapy, not excluding childhood disease.[95] The introduction of isoniazid has altered Lincoln's view[67] that primary infection in children is not benefited by specific therapy. The only area in which disagreement exists is in the treatment of the recent tuberculin converter.[95]

What Drugs Should Be Used. There is today no ideal regimen of therapy. It is generally agreed that PAS and SM should not be given alone and isoniazid is usually given in combination with another drug. There is the minority opinion[60] that isoniazid alone is useful under certain conditions, for the development of bacterial resistance to isoniazid does not seem to have the same clinical significance as resistance to streptomycin. Triple drug therapy (INH plus SM plus PAS) seems to have no advantage in the treatment of uncomplicated tuberculosis but may be indicated in the treatment of meningitis.[102]

The Committee on Therapy of the American Trudeau Society, in a statement prepared by Dr. Muschenheim, recommended the following

regimens:[95] (1) SM + PAS: streptomycin, 1.0 gm. intramuscularly two or three times weekly, and PAS, 12 gm. daily by mouth in three or four divided doses. (2) Isoniazid alone, 4 to 5 mg. per kg. of body weight per day in two or three divided doses. (3) INH + SM (daily): isoniazid, 4 to 5 mg./kg./day, and SM 1.0 gm. IM once daily. (4) INH + SM (twice weekly): same dosage as in No. 3 but SM given twice weekly instead of daily. (5) INH + PAS: isoniazid, 4 to 5 mg./kg./day, and PAS, 12 gm. daily. (6) INH + SM + PAS each in dosage as foregoing; streptomycin given daily or twice weekly.

The statement notes that regimens No. 1 and 2 are slightly less effective than the other regimens but the differences are not large. The committee believes, however, that isoniazid should always be included in the therapy of the more dangerous forms of tuberculosis such as miliary, meningeal and acute extensive pneumonic pulmonary tuberculosis.

The dosage suggested are suitable for all forms of tuberculosis except miliary and meningeal. In the treatment of meningitis[102] isoniazid is recommended in the dosage of 8–10 mg./kg./day for the first two to three weeks after which it may be reduced to 4–5 mg./kg. daily. In these forms of tuberculosis it is also recommended that SM be given daily during the first three months of therapy. PAS 12–20 gm. daily is considered optional.

How Long Treatment Should Be Continued. The cooperative trials by the V.A.-Army-Navy proved that long-term chemotherapy was superior to treatment for short periods. It is now suggested that treatment should be continued for at least a year and more often eighteen months or even longer.[95]

If one considers the pathology and bacteriology of tuberculosis, it is reasonable to assume that long-term therapy should be more effective than short terms of treatment. The outcome of infection is determined by the balance between extension and healing. Chemotherapy with accepted drugs does not eradicate infection but it does tend to tip the balance in favor of healing and favors the containment of infection. Clinical observations on untreated tuberculosis indicate that the longer the patient remains well after initial diagnosis and treatment, the less likely is relapse. It is probable that bacilli contained in caseous areas become less and less capable of reactivating infection the longer they remain metabolically inactive. An argument can be made for indefinite prolongation of treatment—or at least treatment might be given during the first two to three years after infection is arrested since this is the period when relapse is most likely to occur. The solution of this problem will require careful analysis of relapse rates with various regimens but treatment for indefinite periods already is being tested.[103]

No matter how long treatment is continued one point seems clear. Chemotherapy of tuberculosis seems to be more effective if therapy is uninterrupted than if intermittent. The choice of a drug regimen should be made carefully and the regimen should then not be stopped unless serious toxicity is manifest or unless the bacilli become resistant. There are good theoretic reasons for this principle. Let us suppose that treatment is being given with isoniazid and streptomycin. A few isoniazid-resistant organisms develop but these are still susceptible to streptomycin.[85] As long as therapy is continued this population of INH-resistant organisms will not multiply. Let us now suppose that therapy is stopped. The INH-resistant organisms may now multiply. If treatment is begun again with INH + SM there may now be a sufficient number of INH-resistant organisms so that some of these will become streptomycin-resistant and, as these organisms resistant to both drugs multiply, both drugs become valueless.

Chemotherapy, Bedrest, Collapse Therapy and Surgery. There is little doubt that chemotherapy has radically changed the treatment of tuberculosis but it has not eliminated the need for other forms of therapy. Bedrest need not be as complete or prolonged but will continue to be indicated until such time that acceptable evidence is presented to the contrary. Collapse therapy and surgery should be fitted to the general therapeutic program of the patient. Certainly, cavities which remain open after maximum improvement on chemotherapy should be excised or collapsed surgically.

THE FUTURE

How close are we to the solution of the tuberculosis problem? Caution was voiced at the start of this article about premature enthusiasm. It is true, however, that the weapons exist today to eliminate tuberculosis as a serious health problem, but these weapons must be used intelligently if the maximum benefit is to be derived. Modern chemotherapy certainly saves

the lives of many who would have died a decade ago, shortens the period of hospitalization necessary for treatment, and makes possible the surgical treatment of cavernous tuberculosis in patients who in the past were comparatively well but always infectious. This means that more beds are available for treatment of active cases. It also means that the number of cases of arrested or inactive tuberculosis in the population is temporarily increased. It would be a great mistake to relax public health measures because we have new and potent drugs. Vigorous case finding programs become all the more important today if the disease is to be completely controlled. Prevention is still far more economical than cure.

The development of new drugs continues and standardized methods for testing antimicrobial agents in cooperative studies makes rapid clinical evaluation possible. The introduction of new potent chemotherapeutic agents will further simplify the treatment of tuberculosis by offering alternative regimens, particularly in retreatment cases.

Continued research is necessary, however, for certain fundamental problems remain unsolved. Relapse of tuberculous infection is largely a problem of host resistance, and little is known about the factors which cause variation in resistance of the host. Cornforth, Hart, Rees and Stock[104] have demonstrated that certain nonionic surface-acting polyoxyethylene ethers have a dramatic suppressive action on experimental tuberculosis although they have no effect on the bacillus *per se*. Mackaness[105] has shown that one such agent (Triton WR-1339) alters the host in such a way that monocytes from a treated animal are able partially or completely to inhibit intracellular tubercle bacilli.

It is obvious that an agent which has its primary effect on host resistance would be a tremendously important adjunct to the antimicrobial therapy of tuberculosis. Such an agent could not be influenced by drug resistance because its effect would be on the host and not on the bacillus. It might be possible to continue treatment with such an agent after traditional chemotherapy had been stopped.[106]

There are other important areas for investigation. If more is understood about the metabolism of the tubercle bacillus under various conditions of growth and inhibitors a more rational approach to therapy can be made. It is particularly important to know more about the altered metabolism of tubercle bacilli *in vivo*, and in what way this changes the action of antimicrobial agents. The change in the metabolism of certain drug-resistant bacilli should be explored further, and related to changes in virulence. Another fertile field for investigation is the mode of action of antimicrobial agents in terms of the biochemical changes which occur within the bacillus. Progress in this field will be closely linked with work on the normal metabolism of tubercle bacilli. Finally, the mode of action of INH-pyrazinamide in eradicating infection in mice is an important field for further research. Since eradication of infection is the ultimate goal of therapy, careful search should be made for other drugs or combinations of drug which will attain this effect.

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The Fate of Tubercle Bacilli *In Vivo**

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In recently published lectures Dubos[1] discussed the microorganisms *in vivo* and called attention to the obvious but frequently ignored fact that microbial cells, on entry into the host, find themselves in an environment significantly different from that which is generally considered "physiologic." The ability of microorganisms to survive and to multiply in the varied physicochemical environments that exist within different tissues determines in large part their ability to produce infection. Moreover, these same environments affect also the ability of phagocytes, antibodies and antimicrobial agents to interfere with the survival and multiplication of organisms within the tissues.

The constantly increasing interest in antimicrobial agents has led recently to an analysis of current knowledge concerning the mechanisms by which these compounds exert their effects *in vivo*, and to consideration of those factors within the host which may interfere with their growth inhibitory action. Possible reasons why antimicrobial agents may fail to sterilize lesions have also been discussed.[1–3] It is difficult, of course, to estimate the degree of bacteriostatic or bactericidal action that may result *in vivo*. Furthermore, one cannot state with certainty the extent to which an antimicrobial agent may be responsible for the effect and the extent to which the resistance of the host may influence the end result. In those instances in which control of an acute infection by an antimicrobial agent has implied that the invading microorganisms have been eradicated by that antimicrobial, actual evidence to this effect has not been forthcoming. Indeed, the evidence, particularly with respect to tuberculosis, has emphasized the fact that it is seldom if ever possible to eradicate every single member of a particular infecting microbial population.

Evaluation of the extent to which antimicrobial agents are capable of eradicating microorganisms present in tissues and body fluids has been handicapped by inadequate methodology. At present, one can measure only the number of microbial cells surviving within the environment of the tissue or fluid in question and capable also of multiplying when inoculated into an arbitrarily selected and artificial culture medium. The available technics do not permit demonstration of the viability of those microbial cells which survive but are unable to metabolize actively, multiply and give off progeny. Failure to demonstrate the presence of living organisms after contact with an antimicrobial agent thus in no way indicates that all of the infecting microorganisms have necessarily been eradicated by that antimicrobial. Only when actively metabolizing cells, capable of growth and multiplication, are apparent can one attach significance to the findings. At such times it must be assumed that those organisms capable of multiplying *in vitro* presumably could also multiply *in vivo* and bring about relapse under appropriate conditions.

The principal purpose of the present communication is to review the extensive literature pertaining to the survival of tubercle bacilli in tuberculous pulmonary lesions. In approaching this subject, however, it is of interest to give brief consideration to the situation as it applies to other infections.

The failure of antimicrobial agents to sterilize has been observed repeatedly. Although the most detailed observations reported thus far have concerned the survival of tubercle bacilli within healed pulmonary lesions, there is nonetheless abundant evidence to indicate that failure to eradicate all infecting microbial cells within tissues or body fluids is a widespread phenomenon.

As early as 1942 Hobby et al.[4] presented evidence to show that penicillin is effective only against actively dividing microbial cells, and in 1944 Bigger[5] reported that penicillin may fail to sterilize large populations of staphylococci *in vitro* because of the presence of so-called "persisters," that is, organisms which are not

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undergoing cellular division and against which therefore, penicillin, is capable of exerting little or no effect. It was suggested by Bigger that in some instances failure to cure staphylococcal infections in man might be due to the presence of similar "persisters" *in vivo*. Such cells were estimated to be few in number, rarely exceeding one per million bacteria. They differ from resistant organisms in that the former on subculture give rise to drug-susceptible progeny while the latter give rise to resistant cells. They differ also in that the former exist in small numbers and merely prevent total sterilization while the latter readily increase in number and also interfere with the degree of bacteriostasis.

Since 1944, failure to sterilize has been observed repeatedly; although no systematic study of this phenomenon has been reported with respect to non-tuberculous infections. It is a well known fact that animals experimentally infected with virulent strains of Streptococcus hemolyticus or Diplococcus pneumoniae, for example, at times develop acute and fatal septicemia within a few days after cessation of penicillin therapy despite the fact that the quantity of penicillin administered was sufficient to prevent development of the infection during the treatment period. Hobby et al.[7] have reported, furthermore, that the administration of large amounts of penicillin promptly after the intravenous inoculation of a penicillin-susceptible strain of Staphylococcus aureus into white mice and throughout a subsequent three-week period failed in most instances to alter the frequency with which infection (that is, non-suppurative pyelonephritis) developed. The failure of penicillin to prevent the development of infection could not be explained on the basis of drug resistance for the invading microorganism was highly susceptible to penicillin as indicated by conventional *in vitro* drug-susceptibility tests and by chemotherapeutic studies of mice with acute septicemic infection.

Failure to eradicate every member of an infecting population perhaps has been demonstrated most effectively in those studies in which microbial population counts have been made on homogenized tissue. In 1950 Werner and Knight[8] reported a detailed study of the effect of chlortetracycline, oxytetracycline, chloramphenicol and neomycin used singly, in combination, or in combination with sulfadiazine, upon the course of a generalized infection produced in black mice by the intraperitoneal inoculation of Brucella melitensis. Although a combination of chlortetracycline and streptomycin proved to be the most effective type of treatment, complete eradication of brucellae and suppression of the lesions of brucellosis were not achieved with any of the drug regimens tested. Similarly, Hobby and associates[9,10] have failed to eradicate Salmonella typhimurium from the spleens and intestinal contents of animals experimentally infected with this species of microorganism. Although a marked decrease in the number of infecting organisms was noted in many instances, large amounts of chloramphenicol administered over prolonged periods of time failed to eradicate all of the salmonella.

One might mention also certain observations made on animals infected with Mycobacterium leprae murium. Although more difficult to analyze, because this organism has not to date been cultivated *in vitro*, population counts, made by a previously described[10] standardized procedure, on spleen homogenates from experimentally infected mice, have indicated that, even after apparently effective chemotherapy, acid-fast microorganisms are invariably detected. Whether or not the residual stainable bacilli in such animals are viable cannot be determined from the evidence available. In this regard it is of interest, however, that after therapy, patients with leprosy likewise continue to harbor stainable acid-fast organisms which cannot be tested for viability because one can neither cultivate Mycobacterium leprae *in vitro* nor transmit the infection from man to animals.

Lastly, attention should be called to the observations of Shaffer, Kucera and Spink[11] who have demonstrated that, when exudative leukocytes containing intracellular brucellae are maintained *in vitro* in a synthetic medium, the intracellular organisms are protected to a considerable extent against the effects of streptomycin and certain other antimicrobial agents present in the surrounding extracellular environment. Moreover, pathogenic (coagulase-positive) staphylococci have been shown to survive for long periods of time after phagocytosis by human polymorphonuclear leukocytes.[12] Once within the phagocytic cells they are protected from the action of the antimicrobial agents, penicillin, streptomycin and bacitracin. Concentrations of these drugs from 50 to 100 times the usual *in vitro* inhibitory concentrations fail to reduce the populations of

staphylococci residing within human polymorphonuclear leukocytes.[13]

Thus it is apparent that under experimental conditions, it is rare that infecting microorganisms are totally eradicated from the environment in which they reside. Less clear-cut evidence is available with respect to the fate of such infecting organisms in man. Numerous observations suggest, however, that the situation with respect to naturally occurring human infections is not unlike that which exists in experimentally induced animal infections.[2]

In 1951 D'Esopo, Ryan and Medlar[15] first reported that acid-fast bacilli could be observed within "closed" or healed tuberculous lesions after long-term chemotherapy but that these organisms failed to grow when cultivated by routine microbiologic procedures. This observation was confirmed in the following year when D'Esopo and his associates[16] reported on eighty-four resected specimens from patients with pulmonary tuberculosis. Most of the patients in this series had received 1 gm. of streptomycin daily in combination with para-aminosalicylic acid during the preoperative period: a few had received 0.5 gm. of streptomycin daily with para-aminosalicylic acid. All were in the non-infectious state at the time of surgery and had multiple cavities which had closed in the course of chemotherapy. Maximum resolution of tuberculous lesions presumably occurred in all patients prior to resection. Mycobacterium tuberculosis was recovered from only four of the thirty-two patients who had received an original course of less than eight months of preoperative chemotherapy, and from none of the twenty-four patients who had received more than eight months of uninterrupted original chemotherapy during the preoperative period. Mycobacterium tuberculosis was recovered, on the other hand, from eight of sixteen patients who had received a short retreatment course (that is, less than eight months) of preoperative chemotherapy, but was recovered from only one of twelve patients whose preoperative retreatment exceeded eight months. Based on these observations, the conclusion was reached by D'Esopo and his associates that chemotherapy and closure or inspissation of necrotic lesions are the two factors that bring about the death of the bacilli "if in fact they are dead."

The observations of D'Esopo and his associates were further confirmed by simultaneously published data reported by Hall.[17] This investigator succeeded in recovering strains of M. tuberculosis from only one of the eleven patients who had received an uninterrupted original course of preoperative chemotherapy for one to six months and from four of eight individuals who had received retreatment courses of preoperative chemotherapy during periods ranging from one to six months. In 1953 and 1954 additional data were presented by D'Esopo et al.,[18] by Hall and his associates[19] and by various other investigators[20–29] which emphasized further the difficulties attendant upon the recovery of tubercle bacilli by orthodox microbiologic technics from "closed" pulmonary lesions after long-term chemotherapy. (Tables I and II.)

The question was raised by Dubos,[30] in 1952, as to whether or not the technics employed were adequate to prove conclusively the non-viability of microorganisms and he further suggested the possibility that physicochemical factors within necrotic lesions might suppress the metabolism of residual surviving microorganisms, thus making difficult the demonstration of their viability. Tarshis,[31] furthermore, called attention to the fact that tubercle bacilli could be recovered from sputum and gastric specimens as late as four months after the start of incubation and suggested that current technics for the cultivation of tubercle bacilli should be re-evaluated.

Microbiologic observations of tuberculous lesions studied both morphologically and culturally during an incubation period of six to nine months were subsequently presented in a series of reports by Hobby, Auerbach and their associates.[32–35] Four basic differences exist between the technic utilized by these investigators and the technics which had been used previously.

1. All specimens were frozen immediately after surgery and were not thawed until just prior to dissection and cultivation of the lesions.

2. Since saline solution as well as distilled water are known to be deleterious to many bacteria, the tissue was blended and homogenized in a balanced liquid medium containing a sufficient quantity of albumin to permit neutralization of certain of the toxic components of the necrotic material.

3. In order to facilitate neutralization of the toxic components of the necrotic material, the homogenate was centrifuged and the sedimented

TABLE I

MICROBIOLOGIC OBSERVATIONS ON RESECTED HUMAN PULMONARY LESIONS

Author	Duration of Preoperative Chemotherapeutic Regimen (mo.)	No. of Resections in Which Mycobacterium Tuberculosis Was Detected/Total No. Studied					
		Original Treatment		Retreatment		All Cases	
		By Microscopy	By Culture and/or Guinea Pig Inoculation	By Microscopy	By Culture and/or Guinea Pig Inoculation	By Microscopy	By Culture and/or Guinea Pig Inoculation
* Vandiviere et al.[20]	4–<8	.....	.....	..	.....	5/5	0/5
	8–<12	.....	.....	..	.....	3/3	0/3
	12–<24	.....	.....	..	.....	2/2	0/2
	Total	.....	.....	..	.....	10/10	0/10
*Steele[21]	4–8	14/23	1/23	..	1/5	.....	2/28
	8–12	37/55	3/55	..	3/9	.....	6/64
	12–18	26/45	6/45	..	1/5	.....	7/50
	18–24	2/2	0/2	..	0/2	.....	0/4
	Total	79/125	10/125	..	5/21	.....	15/146
† Larson et al.[22]	0–2	.....	.....	..	.....	17/21	7/21
	2–4	.....	.....	..	.....	14/15	4/15
	4–6	.....	.....	..	.....	6/10	0/10
	6–>6	.....	.....	..	.....	1/3	0/3
	Total	.....	.....	..	.....	38/49	11/49
* Falk et al.[23]	0–4	.....	5/22	..	1/3	.....	6/25
	4–8	.....	1/22	..	1/9	.....	2/31
	8–12	.....	0/8	..	2/10	.....	2/18
	>12	.....	0/4	..	0/5	.....	0/9
	Total	.....	6/56	..	4/27	.....	10/83
‡ D'Esopo et al.[18]	0–4	.....	1/7	..	2/11	.....	3/18
	4–8	.....	6/17	..	8/39	.....	14/56
	8–12	.....	0/6	..	2/29	.....	2/35
	>12	.....	1/8	..	2/25	.....	3/33
	Total	.....	8/38	..	14/104	.....	22/142

* In view of uniform agreement that M. tuberculosis may be recovered almost invariably from open cavities, only those data which apparently pertain to "closed" or "non-airbearing" cavities have been included herein. The designation was not always clear but when the author specifically designated a cavity as an open one it was omitted from the present summary table.

† The data presented by Larson and his associates[22] do not permit interpretation of the exact number of open cavities studied. All data reported by these investigators have been included herein.

‡ A total of 219 lesions were removed for study from 104 resected specimens from patients who had received an original course of preoperative chemotherapy. Of these 219 lesions five were open cavities and from two of the five M. tuberculosis was recovered by culture and/or guinea pig inoculation. Ninety lesions were removed for study from thirty-eight resected specimens removed from patients who had received interrupted courses of chemotherapy prior to surgery. Eight of the ninety lesions were open cavities while six of the eight revealed the presence of M. tuberculosis by culture and/or guinea pig inoculation.

TABLE II

MICROBIOLOGIC OBSERVATIONS ON RESECTED HUMAN PULMONARY LESIONS

Modified from:	Duration of Preoperative Chemotherapy (mo.)	Preoperative Chemotherapeutic Regimens					All Regimens
		SM 1 Gm. Daily with PAS	SM Twice Weekly with PAS	SM 0.5 Gm. Daily with PAS	INH Alone or Combined	Mixed	
		No. of Resections in Which M. Tuberculosis Was Detected by Culture and/or Guinea Pig Inoculation/Total No. Studied					
Steele[21]	Original chemotherapy	.....	.....	....	.....	....	.....
	4–8	1/16	0/6	....	.....	0/1	1/23
	8–24	2/33	3/39	....	.....	4/30	9/102
Larson et al.[22]	All cases	.....	.....	....	.....	....	.....
	0–4	.....	.....	....	11/36	....	11/36
	4–>6	.....	.....	....	0/13	....	0/13
*Falk et al.[23]	Original chemotherapy	.....	.....	....	.....	....	.....
	0–4	0/4	2/13	2/5	3/5	....	7/27
	4–8	1/8	6/17	0/2	0/3	....	7/30
	8–12	0/4	0/4	0/1	0/0	....	0/9
	>12	0/3	0/1	0/0	0/0	....	0/4
†D'Esopo et al.[18]	Original chemotherapy	.....	.....	....	.....	....	.....
	0–4	2/8	0/0	0/3	.....	....	2/11
	4–8	1/18	3/6	4/15	.....	....	8/39
	8–12	0/15	2/6	0/8	.....	....	2/29
	>12	0/17	1/5	1/3	.....	....	2/25
All investigators[18,21–23]	0–4	2/12	2/13	2/8	14/41	0/0	20/74 (27%)
	4–8	3/42	9/29	4/17	0/16	0/1	16/105 (15%)
	8–>12	2/72	6/55	1/12	0/0	4/30	13/169 (8%)
	Total	7/126	17/97	7/37	14/57	4/31	49/348 (14%)

* Of these seventy resections only fifty-six apparently were "closed" lesions. Ten of these fifty-six showed the presence of M. tuberculosis by culture and/or guinea pig inoculation. With respect to patients who had received a retreatment course of preoperative chemotherapy, "There was little difference between the percentage of findings in this group, divided according to duration of therapy for eight months or more."

† As indicated in Table I, five of the 219 lesions from these 104 resected speciments were open cavities. Three of 129 "closed" lesions from patients who had received 1 gm. of streptomycin daily with PAS revealed M. tuberculosis by culture and/or guinea pig inoculation. Five of sixty "closed" lesions from patients who had received 0.5 gm. of streptomycin daily with PAS and six of twenty-five from patients who had received 1 gm. of streptomycin twice weekly with PAS also revealed M. tuberculosis on culture and/or guinea pig inoculation.

fraction washed three times with fresh quantities of the albumin-containing medium.

4. All cultures were incubated for six to nine months at 37°C. in a medium that does not deteriorate under such conditions.

M. tuberculosis was recovered from forty-eight of seventy-five lesions, or from 64 per cent, of the lesions studied by this group of investigators. Tubercle bacilli were recovered from thirty-one of forty-three, or 72 per cent, of the resected specimens included in the series. No correlation was noted between the frequency with which viable cells were detected and (1) the duration of the preoperative period of non-infectiousness, (2) the duration of preoperative chemotherapy, (3) the total duration of chemo-

therapy, (4) the preoperative chemotherapeutic regimens employed or (5) the morphologic nature of the lesion. (Tables III and IV.)

Seventy-three of the seventy-five lesions included in the studies of Hobby, Auerbach et al.[35] were morphologically "closed" or healed lesions; only two were open cavities. Six of the seventy-five lesions had been removed from five patients who had received no preoperative chemotherapy. Acid-fast bacilli were detected on microscopy in sixty-one of the seventy-five lesions. The organisms were sparse, however, a fact which is consistent with the observations of Canetti[36] who has noted that the number of stainable bacilli diminishes as healing progresses. Tubercle bacilli emerged only in a portion of the culture tubes and/or guinea pigs used for study of each lesion. M. tuberculosis was recovered from eleven lesions only after prolonged periods of incubation and in two instances the organisms emerged only after incubation of the homogenized tissue for periods in excess of 223 and 240 days, respectively.

Mention has been made previously that Tarshis[31] in 1952 called attention to the increased frequency with which tubercle bacilli may be recovered from sputum and gastric specimens when the incubation period is prolonged beyond the customary period. Vandiviere et al.[20] have emphasized the same point while Jones and Gentry[37] recently reported that, in a study of 1,258 cultures. M. tuberculosis was recovered from twenty-three specimens only after an incubation period of twelve weeks.

TABLE III*

ANALYSIS OF MICROBIOLOGIC OBSERVATIONS ON RESECTED HUMAN PULMONARY LESIONS

Chemotherapy	No. from Which M. Tuberculosis Was Recovered/Total No. Studied	
	Patients	Lesions
Original	17/26	31/48†
Retreatment	10/12	13/21†
No chemotherapy	4/5	4/6
Preoperative regimens		
SM twice weekly with PAS	10/15	20/30
SM twice weekly with isoniazid	7/10	9/16
SM twice weekly with PAS and isoniazid	5/6	8/10
Isoniazid with PAS	6/8	8/15
No chemotherapy	3/4	3/4

* Modified from Hobby, Auerbach, Lenert, Small and Vaughan, Transactions of the Fourteenth Conference on the Chemotherapy of Tuberculosis, held under the auspices of the United States Veterans Administration, Army and Navy, February 1955. To be published.

† Each series includes one open cavity which contained viable tubercle bacilli.

TABLE IV*

ANALYSIS OF MICROBIOLOGIC OBSERVATIONS ON RESECTED HUMAN PULMONARY LESIONS

Duration in Months	No. from Which M. Tuberculosis Was Recovered/Total No. Studied	
	Patients	Lesions
Preoperative period of noninfectiousness		
0–<3	3/5	4/6
3–<6	11/15	18/26
6–<9	13/16	10/30
9–12	2/3	3/5
24–>24	2/4	3/8
Total	31/43	48/75
Preoperative chemotherapy		
0–<4	6/8	7/11
4–<8	11/16	18/28
8–<12	10/12	19/24
12–<24	4/5	4/8
24–>24	0/2	0/4
Preoperative chemotherapy in original treatment patients		
0–<4	1/2	2/4
4–<8	6/9	13/18
8–<12	7/9	12/16
12–<24	3/4	3/5
24–>24	0/2†	0/4
Total chemotherapy		
0–<4	4/6	5/8
4–<8	10/13	17/24
8–<12	9/12	15/21
12–<24	7/9	9/16
24–>24	1/3	2/6

* Modified from Hobby, Auerbach, Lenert, Small and Vaughan, Transactions of the Fourteenth Conference on the Chemotherapy of Tuberculosis, held under the auspices of the United States Veterans Administration, Army and Navy, February 1955. To be published.

† Two resections from a single patient after twenty-seven and thirty-four months of uninterrupted chemotherapy.

Seven cultures from an additional three patients showed growth of M. tuberculosis only after an incubation period of five months. The latter patients were under treatment with isoniazid. The authors thus concluded that the use of isoniazid has caused a retardation of the growth of tubercle bacilli and suggested that re-assessment of the period of incubation is warranted.

It is of interest that the vast majority of strains of M. tuberculosis isolated by Hobby and her associates[35] was drug-susceptible. A few, however, were resistant to isoniazid, a few were resistant to streptomycin and a few others were resistant to both drugs. From four lesions atypical acid-fast bacilli, showing varying degrees of chromogenicity, were recovered. With the exception of those strains resistant to isoniazid, all were virulent for guinea pigs although in many instances tuberculous disease had not been produced on direct inoculation of the freshly resected tissue.

Based on these observations, it was concluded by Hobby, Auerbach and their associates[32–35] that tubercle bacilli can survive in healed or semi-healed necrotic pulmonary lesions even after prolonged chemotherapy, and that in many instances their viability can be demonstrated by appropriate technics. Undoubtedly not all of the bacilli present in these lesions are viable, they state, but a portion is alive, they can be cultivated *in vitro*, presumably could multiply *in vivo* and bring about relapse under appropriate circumstances.

Observations in experimentally infected tuberculous animals have offered abundant support for this concept.

The physicochemical factors within "closed" or healed tuberculous lesions which might suppress the metabolic processes of residual surviving bacilli have been discussed in detail elsewhere.[33] Comment has been made also concerning observations which suggest that adaptive changes in the enzyme mechanisms within microorganisms may take place *in vivo*, allowing for their survival within the host but preventing their subsequent multiplication either *in vivo* or *in vitro* until an appropriate reversal mechanism has been imposed.

Abundant evidence exists to indicate that in experimental tuberculous infections chemotherapy does not result in eradication of the tubercle bacilli from the animal host although the fact that the invading microorganism are presumably more readily available to attack by the antimicrobial than when situated within necrotic lesions. Feldman and his associates[38,39] were first to demonstrate that tubercle bacilli may persist in organs of infected animals which have completely healed under treatment with streptomycin. This phenomenon was observed subsequently by others;[40–45] and Hobby and her associates[46] have reported that extensive pulmonary disease invariably can be noted at the end of a six-months' period of observation in mice infected intracerebrally with the H37Rv strain of M. tuberculosis and treated over the first thirty-day period following infection with adequate quantities of streptomycin to allow survival of all animals. Although capable of increasing survival time, streptomycin fails to eliminate the infecting organisms from the animal host and fails also to prevent latent development of the disease. M. tuberculosis in like manner persists and ultimately produces tuberculous disease in mice similarly infected and treated with protective doses of oxytetracycline or viomycin.[47,48] These observations in mice are in close agreement with those presented by Steenken and Wolinsky[49] who observed that, although streptomycin and viomycin markedly retarded the progression of tuberculous infection in experimentally inoculated guinea pigs, animals treated with these antimicrobial agents showed small tuberculous abscesses at the original site of inoculation (the inguinal region).

Microbiologic observations on the tissues of surviving drug-treated animals have further emphasized the inability of streptomycin and isoniazid to eradicate tubercle bacilli from the host.[50] Using streptomycin and isoniazid singly and in combination in mice and guinea pigs experimentally infected with the Vallée bovine strain and with the H37Rv human strain of M. tuberculosis, data obtained by direct culture of lung tissue and by guinea pig inoculation of lung and/or spleen homogenates have indicated that viable tubercle bacilli persist during therapy in the majority of instances. One hundred per cent of animals in which treatment with streptomycin was initiated on the day of infection harbored viable tubercle bacilli at the time of sacrifice, while strains of M. tuberculosis were recovered from 44 per cent of those animals receiving isoniazid alone or in combination with streptomycin.[50]

The striking observations of McCune, Tompsett and their associates[51,52] lend further sup-

port to this theory. In a quantitative study of the alterations in the bacterial population effected by antituberculous drugs and drug combinations, these investigators have demonstrated that there is a stable census in the spleen of infected animals despite intensive antimicrobial therapy. This persistence of viable bacilli in the spleen occurs even after prolonged therapy with isoniazid, streptomycin and para-aminosalicylic acid, administered singly, in pairs or all together. Moreover, these "persisters" cannot be accounted for by the emergence of strains of tubercle bacilli which are drug resistant in the customary sense. Pyrazinamide alone produces a sharp reduction below the level of detectability at eight weeks in the microbial population within the spleens of infected animals but this effect is temporary, and a rise in the census occurs in some animals by the twelfth week of infection. A more permanent effect is noted when pyrazinamide is administered concomitantly with any other antituberculous drug, and preliminary observations originally suggested that this drug combination might possibly represent an eradicative type of therapy for tuberculosis. More recent observations[53] have indicated, however, that tubercle bacilli are present in the spleens of animals sacrificed ninety days after cessation of pyrazinamide-isoniazid therapy, thus indicating the persistence of organisms during and after the treatment period. This represents the most quantitative and definitive experimental evidence to date that chemotherapy fails to produce a uniformly eradicative effect.

It is not unreasonable to assume that the failure of the antituberculous drugs to eradicate tubercle bacilli *in vivo* may be related in part to quantitative differences in the ease with which they (i.e., the drug) traverse cell boundaries, the degree of activity which they can exert in an intracellular environment[53–56] and in the biochemical environment of the necrotic tissues.

The welter of observations with respect to the survival of tubercle bacilli in healed pulmonary lesions has made difficult the interpretation of results. Fundamentally different technics have been utilized in the various studies described, and widely differing methods of recording and analyzing the data have been employed. Few investigators have discussed in detail the morphologic nature of the individual lesions studied. Only in the reports from one laboratory[34] are the microbiologic data presented in terms of the individual lesion concerned. In addition, tubercle bacilli may be recovered from some and not from other "closed" lesions within a single specimen,[21] and the organisms may be irregularly distributed throughout different parts of a single lesion.[36]

The question thus arises: What areas of agreement are there among these widely varying sets of data?

It is agreed that tubercle bacilli can be recovered with ease from essentially all open cavities.[36] There is also complete agreement that the number of stainable bacilli within a lesion frequently diminishes as healing progresses, and cultivation of those organisms existing within "closed" or healed lesions, if such a thing can be accomplished, is more difficult than is the cultivation of such organisms from open cavities.[15,16,18,33,36]

Those investigators[33] who have succeeded in cultivating tubercle bacilli from a high proportion of "closed" or healed lesions have stated that "undoubtedly not all of the bacilli are viable," while others[58] have commented that certainly not all but only the major portion of the organisms are dead. Thus there is full agreement that small numbers of "persisters" may exist within these lesions. That these "persisters" can be cultivated *in vitro* has been indicated by the observations of Hobby, Auerbach et al.[32–35] Moreover, their progeny can multiply and produce tuberculosis in guinea pigs. Presumably, therefore, they could multiply in man also and bring about relapse under appropriate conditions.

The basic questions therefore are: In what proportion of active tuberculous lesions which regress to a satisfactory stage of healing under chemotherapy do tubercle bacilli persist? When any single microbiologic procedure, capable of detecting these "persisters" with a high degree of frequency is employed, from what types of patients and/or lesions can they be recovered? Under what conditions do tubercle bacilli survive?

The observations of D'Esopo and his associates[15,16,18] were confined to those residual lesions resected from patients meeting "target point" criteria, defined by the authors as closure of all cavities, consistently non-infectious status for "several" months and roentgenographic stability. The patients whom they selected for resection were mostly those who had been treated "with an arbitrary period of four months

of preoperative chemotherapy." It has been pointed out by these investigators that the frequency with which viable tubercle bacilli may be demonstrated in lesions from patients who have had prolonged chemotherapy is lower in those who receive daily administration of streptomycin than in those who receive it twice weekly.

As evidenced by the data reviewed herein, no correlation has been noted thus far between the frequency with which M. tuberculosis has been recovered and the total duration of chemotherapy, the duration of the preoperative chemotherapeutic period, the chemotherapeutic regimens used or the period of non-infectiousness prior to surgery. The failure to demonstrate a correlation between the viability of tubercle bacilli within "closed" lesions and the type or amount of chemotherapy administered is not surprising. Indeed, as mentioned by D'Esopo et al.[16] in 1952 and Canetti[36] in a study antedating the chemotherapeutic era observed also that M. tuberculosis generally cannot be cultivated from "closed" cavities.

It is not within the scope of the present communication to discuss the clinicopathologic aspects of the problem. It should be pointed out, however, that Hobby, Auerbach et al.[35] have recently presented preliminary data suggesting that factors occurring prior to institution of drug therapy possibly may influence the ability of tubercle bacilli to survive within "closed" lesions.

The observations with respect to tuberculosis offer striking evidence of the fact that it is difficult, perhaps impossible in most instances to eradicate every member of an infecting microbial population by means of chemotherapy. In experimental situations in which the size and portal of the infecting dose as well as the drug regimens used may be manipulated with ease, sterilization unquestionably can be achieved at times. In infections in man, however, especially in those infections in which the organisms are strategically located, as within an area of necrosis, the situation is more complex. To develop eradicative types of chemotherapy effective in any situation is the challenge today.

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Chemotherapeutic and Antibiotic Drugs in the Management of Infections of the Urinary Tract*

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INFECTIONS of the urinary tract are second only to infections of the respiratory tract in frequency but satisfactory information on the incidence, pathogenesis and natural history of pyelonephritis and related infections of the urinary tract is surprisingly incomplete. Certain aspects of the disease are reasonably well established, and these may be summarized briefly:

1. Pyelonephritis is the most common renal lesion found at autopsy, and occurred in 15 to 20 per cent of autopsies performed at large general hospitals.[1-3] In about one-third of the cases of pyelonephritis found at autopsy the lesion was of major importance as a cause of death and this relationship has not diminished significantly since the advent of antibiotics.[4]

2. Chronic pyelonephritis is a more frequent cause of Bright's disease than is chronic glomerulonephritis[2] and is the most common renal lesion in uremia.[5] Acute exacerbation of pyelonephritis is considered to be the common precipitating factor in uremia.[6]

3. Chronic pyelonephritis appears to be etiologically related to malignant hypertension,[1-3,6] and there are strong, but not unchallenged, indications of an etiologic relationship to benign hypertension.[1,2]

4. Pyelonephritis occurs about four times as often in diabetics who have come to autopsy as in non-diabetics, a difference that may reflect the seriousness of the infection in diabetics as much as an increased incidence.[7] Pyelonephritis is also one of the most common complications of pregnancy and occurs in about one-fifth of the cases of toxemia of pregnancy.[8]

These and many other considerations emphasize the importance of pyelonephritis in clinical medicine. It is the purpose of this review to present briefly some of the clinical and laboratory considerations in the management of the bacterial aspects of pyelonephritis and related infections of the urinary tract. No attempt will be made to provide a complete review, and there will be no discussion of tuberculous, gonococcal or rare and unusual infections of the urinary tract. Consideration will be given only to infections of the kidney, ureter and bladder.

Certain generalizations provide a working basis for approaching the management of infections of the urinary tract and will be stated briefly:

1. Symptoms referable to the lower urinary tract, such as dysuria, frequency and urgency, are more common in pyelonephritis than are flank pain, fever, chills and other generalized evidence of sepsis.

2. Whether cystitis or pyelitis occurs without renal involvement is problematic, and the present study will not attempt to go into this question in detail. It will be assumed that in the absence of reliable means for distinguishing "pure" cystitis from cystitis with renal involvement, all patients should be treated as though the latter were present.

3. Regardless of the route (hematogenous, lymphogenous, ascending urogenous) by which bacteria reach the kidney, abnormalities of the urinary tract predispose to chronic and recurrent pyelonephritis. However, a reasonably large number of patients with chronic pyelonephritis have no demonstrable abnormalities of the urinary tract.

4. Chronic pyelonephritis may be present,

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and may heal, without giving rise to symptoms or signs, or even obvious pyuria.

QUANTITATIVE DIAGNOSTIC CONSIDERATIONS

The symptoms, signs and abnormal urinary elements that occur in pyelonephritis are reactions to the presence of bacteria in the kidney. That bacteria may be present in abundance in the urine without giving rise to pyuria or to symptoms[9] only emphasizes the chronic and insidious nature of pyelonephritis in many cases. It suggests that the search for the presence of infection should take as its focus the presence of bacteria, and not findings that are secondary to inflammation or renal damage.

The detection of bacilluria poses certain problems. Manifestly, urine obtained in the usual manner by catheterization of females, or by clean voided mid-stream specimens in males, may yield positive cultures if but a few contaminating organisms have fallen into the urine. However, detailed studies of the growth in urine of common pathogens of the urinary tract show that bacterial multiplication in urine is rapid and that bacterial counts rise to more than 10^8 bacteria per ml. of urine within eight to twelve hours after the inoculation of small numbers of bacteria.[10] Thus the discharge of even a few bacteria from the kidney may lead rapidly to high bacterial counts in the urine. When bladder urines from patients with unquestioned acute pyelonephritis were examined quantitatively, none contained less than 100,000 bacteria per ml. Urine obtained from the renal pelvis of some patients with renal infections have been found to contain fewer than 10,000 bacteria per ml. when the bladder urine, obtained at the same time, contained more than 10^8 organisms per ml. When female patients with no clinical evidence of pyelonephritis were catheterized, their bladder urines were found to be free of bacteria in about 25 per cent of cases, to contain less than 100 bacteria per ml. in about 40 per cent more, and to contain less than 1,000 bacteria per ml. in about 30 per cent more.[10] The number of patients with bacterial counts greater than 10^5 bacteria per ml. has varied according to the groups of individuals studied, being as high as 25 to 30 per cent in asymptomatic, diabetic women,[11] and negligible in young children with no abnormality of the urinary tract.[12]

It is thus apparent that true bacilluria is probably present when the bacterial counts are 10^5 or more per ml. of urine, and that counts significantly lower than this probably represent contamination. The limitations of this quantitative approach to the diagnosis of significant bacilluria are not many. Bacterial counts may fall below 10^5 bacteria per ml. of urine in the presence of active pyelonephritis when:

1. A bacteriostatic agent is in the urine.
2. The rate of urine flow is rapid, the numbers of bacteria discharged from the kidney small, and pooling of the urine in the bladder for a long enough time to permit multiplication to significant levels has not occurred. It is for this reason that the first morning specimen, representing the longest possible incubation time for urine in the bladder, is preferable to random specimens.
3. Urinary pH and dilution have limited the degree of bacterial multiplication in urine. The rate of multiplication of the most common pathogens of the urinary tract is not markedly affected until the urinary pH has fallen below 5.5 and bacteriostasis does not occur until pH values of less than 5.0 have been obtained. Dilution effects become noticeable at about specific gravity of 1.003 and then only in pH ranges below 5.5. Alkaline urines have little effect on bacterial survival unless the pH is above 8.5, which is not likely to be encountered even after pronounced urea-splitting.[10]
4. Fastidious organisms that grow poorly in urine are encountered. This is rare in infections of the urinary tract but occasionally such organisms as group A streptococci, some Streptococcus viridans strains, occasional enterococci or staphylococci, anaerobic streptococci, etc. may grow poorly in urine and may be present in but small numbers during active infection. If an organism has been isolated from cultures of urine and is thought to be a urinary tract pathogen, even though it is present in small numbers, it can be inoculated into sterile urine and its habit of growth observed. The presence of organisms that grow poorly in urine, but can be isolated in small numbers on other media, would be consistent with the possibility that such organisms were urinary tract pathogens in the patient under study.
5. In obstruction of the ureter, bladder urine is free of bacteria. In fact, absence of bacilluria when symptoms and signs suggest renal infection, should suggest the possibility of ureteral obstruction. Occasionally, as a consequence of hematogenous dissemination of such organisms as staphylococci, abscesses may be produced in

the kidney which do not discharge organisms into the tubules. Usually these are associated with manifest clinical evidence of sepsis and renal localization. Indeed, the discharge of pyogenic organisms into the urine in such cases may coincide with lysis of fever and resolution of the active process.[13]

percentages of staphylococci, enterococci,* and members of the genera Proteus, Aerobacter and Pseudomonas increase in chronic and complicated infections of the urinary tract, and particularly those that have occurred following instrumentation, catheterization or prior antibacterial therapy. (Table 1.) Hence statements

TABLE 1

INCIDENCE OF PATHOGENS IN UNSELECTED PATIENTS WITH INFECTIONS OF THE URINARY TRACT

Type of Infection	Coleman and Taylor[16]		Erlanson and Jönsson[17]	
	60 Uncomplicated Cases (%)	40 Complicated Cases (%)	125 Uncomplicated Cases (%)*	1,087 Complicated Cases (%)
E. coli	82	18	78	43
Paracolon	5	5		
Atypical coli	5	5		
A. aerogenes	2	45		
P. vulgaris	5	43	4	10
P. morgani	0	35	0	0
Ps. aeruginosa	0	7	1	7
Miscellaneous rods	2	0	0	0
Enterococcus	18	25	14	31
Staphylococcus			4	9

* Some of these are chronic or recurrent infections, but no complicating lesion of the urinary tract was demonstrable.

For practical clinical purposes the Gram stain of the freshly collected and unsedimented urine will differentiate contamination from infection, since organisms are readily found in stained specimens of urine when about 10^5 bacteria or more per ml. are present.[10,14]

It is apparent that in any quantitative consideration of urinary flora the care used in obtaining the specimens and the rapidity with which the specimens are processed after they have been obtained become limiting factors. The use of the Gram stain as a guide to the quantitative evaluation of the urinary flora is no more valid than the technical skill with which the slides are prepared and interpreted.

FREQUENCY OF OCCURRENCE OF PATHOGENS

The frequency of occurrence of any given bacterial species as a pathogen in the urinary tract is, to a large degree, a function of the clinical material under examination. Although Escherichia coli and closely related bacteria are the most common bacteria found in most series of acute and chronic pyelonephritis, the relative of the incidence of various bacterial species in a given series of cases reflect the type of disease and the previous treatment of the patients involved. Similarly, single bacterial species are found in the urine in 80 to 100 per cent of instances of acute uncomplicated pyelonephritis whereas in infections complicated by structural abnormalities of the urinary tract the incidence of pure cultures may be as low as 20 per cent.[16]

The effectiveness of a given therapeutic approach depends upon many factors, the best recorded of which are the sensitivity of the pathogens involved to the therapeutic agents, the effective concentration of drug at the site where the bacteria occur, the presence of structural

* The term enterococci will be used in this paper to denote the ill-defined streptococci that are resistant to heating to 56°C. for thirty minutes, and that usually have certain other distinguishing properties such as the capacity to resist strong salt solutions, to ferment mannitol, etc. The term includes most group D streptococci, and the species characterized as Streptococcus fecalis. The problems of precise definition of these streptococci have been discussed in detail by Nyman.[15]

abnormality of the urinary tract, and the degree of chronicity of the infection.

SENSITIVITY OF BACTERIA TO THERAPEUTIC AGENTS

Acidification, Ketone Bodies, Mandelic Acid, Methenamine Mandelate. Earliest attempts at medical therapy were directed toward rendering the urinary tract unsuitable for bacterial multiplication by inducing changes in pH and specific gravity. These were of limited value because pH values below 5.0 are necessary to kill most of the common pathogens of the urinary tract, and because extreme dilution of the urine inhibits bacterial multiplication but slightly.[10,18] The discovery that beta-hydroxybutyric acid and a variety of other organic acids, including mandelic acid, inhibit bacterial multiplication in acid urines was a major advance in therapy.[19,20] The use of ketogenic diets and acidifying salts gave way to mandelic acid and methenamine mandelate when these substances were demonstrated to be at least as effective as ketogenic diets and less troublesome to use. The successful use of mandelic acid and methenamine mandelate depends upon maintenance of concentrations in urine of 0.5 to 1.0 per cent of drug at pH less than 5.5. Activity of the drugs decreases markedly as the pH increases. Most strains of the common urinary tract pathogens (E. coli, Aerobacter aerogenes, Proteus, Pseudomonas aeruginosa, Staphylococcus aureus and S. fecalis) are inhibited *in vitro* by mandelic acid and methenamine mandelate in concentrations of 0.03 to 1.0 per cent but A. aerogenes and Proteus tend to be more resistant than the others.[21]

TABLE II

EFFECT OF VARIOUS URINARY ANTISEPTICS ON INFECTIONS OF THE URINARY TRACT

Reference	Treatment	Etiologic Agent	Bacteriologic Control* (%)	Remarks†
22	None or phenyl salicylate		73	Presumably acute, uncomplicated infections
23	Neoarsphenamine	Staphylococcus	52	Poor effect on E. coli and S. fecalis
24	Bacteriophage		36	
25	Ketogenic diet	E. coli	85	Some chronic infections
26	Methenamine		55	Acute uncomplicated infections
26	Methenamine		33	Chronic infections
27	Mandelic acid	E. coli	86	
28	Mandelic acid	Various	85	
29	Methenamine mandelate	E. coli	65	
30	Methenamine mandelate	E. coli	86	80 per cent of these were acute, uncomplicated
30	Methenamine mandelate	Staph. aureus	71	
31	Acidification	Various	3	All chronic, uncomplicated infections of many years' duration
31	Acidification + antiseptics	Various	20	

* The term "control" rather than "cure" will be used throughout because of the variability of follow-up study. Criteria for control vary from a single negative urine culture to multiple negative smears and cultures. Clinical criteria alone were not included in the evaluation.

† Infections classified as acute uncomplicated, chronic (constant bacteriuria or recurrences more than twice); complicated (demonstrable abnormalities of the urinary tract).

The clinical effectiveness of a variety of methods of treatment is shown in Table II. The spontaneous resolution of acute uncomplicated infections of the urinary tract is undoubtedly high, yet there is good reason to believe that the ketogenic diet, mandelic acid, methenamine mandelate, etc. constitute effective therapy. Thus, Hecht-Johansen and Warburg[32] in 1926 treated forty patients with acidifying salts and urinary antiseptics and found that the urine of twenty-four of these became sterile, all of the latter patients had uncomplicated infections. By contrast, eleven of the sixteen patients in whom bacilluria persisted had demonstrable anatomic changes in the urinary tract. Similar data have been recorded by many other workers. The introduction of the ketogenic diet increased the percentage of bacteriologic control in acute uncomplicated infections; some patients with prolonged bacilluria and demonstrable urinary tract abnormalities were freed of their

urinary tract infections, although relapses were frequent and probably occurred in at least one-third of the patients who were discharged as free of infection.[33]

Mandelic acid provided effective bacteriologic control in eighty-nine of 105 patients with urinary tract infections, most of whom had chronic infections. However, fourteen of the sixteen failures were complicated by some abnormality of the urinary tract, and there was an over-all relapse rate, during the subsequent one to three years, of about 30 per cent.[28] Essentially the same situation occurs following the use of methenamine mandelate.[30]

It was repeatedly observed in the earlier literature that urinary tract infections due to E. coli were the most readily controlled by the various treatments, that staphylococcal infections also responded well, but that aerobacter or proteus infections were less likely to be controlled. When cultures of the urine showed more than one pathogen to be present the results were often disappointing even when the organisms in the mixture were susceptible ones, such as E. coli and staphylococci. The explanation lies in part in variation in susceptibility of microorganisms to the antibacterial measures and in part in the well established finding that mixed cultures, and organisms of the Proteus, Aerobacter or Pseudomonas genera, are more likely to be found in chronic and complicated infections than in acute, uncomplicated ones. (Table I.)

The advantages of mandelic acid and methenamine mandelate are their ease of administration, relative lack of toxicity and effectiveness *in vitro* against a broad range of bacteria. Bacilluria may be partially suppressed for prolonged periods of time in patients with chronic infections, without cure but with symptomatic relief and without the emergence of resistant variants of the pathogens.[14]

The disadvantages of these drugs are the appearance of hematuria and gastrointestinal symptoms if the critical doses are exceeded (about 12 gm. per day of mandelic acid or about 6 gm. per day of methenamine mandelate). These drugs are entirely dependent for their effect on proper acidification of the urine.[21] This explains the frequent failures when urea-splitting bacteria such as proteus cause infections and, of course, makes successful therapy difficult in patients with impaired renal function.

It is noteworthy that despite the relative lack of effectiveness of mandelic acid and methenamine mandelate at body pH values, eradication of urinary tract infections is possible. This suggests that preoccupation with effective drug concentrations at "tissue" in contras to "urinary" levels may not always be warranted.

Sulfonamides. The value of sulfonamides in the treatment of urinary tract infections is well established, and the introduction and re-introduction of sulfonamide derivatives to the market attests to the continued interest of the medical profession in their use. Some characteristic experiences with various sulfonamides are summarized in Table III. The substituted sulfonamides were introduced in order to broaden the effective action of sulfanilamide to include more strains of such organisms as pneumococci, staphylococci, gonococci and others. In the course of development of various derivatives some increase in range of action against common urinary pathogens was also observed. However, the effectiveness of sulfanilamide in sterilizing the urine in most acute uncomplicated E. coli infections of the urinary tract is probably as great as that of its derivatives. The advantages of the derivatives in the treatment of urinary infections lie in their broader range of action (particularly with respect to staphylococci and members of the Proteus and Pseudomonas groups), their greater activity, and their lesser toxicity when compared with sulfanilamide. Only a few attempts at critical comparison of the antibacterial potency of the various clinically useful sulfonamide derivatives have been made, even under arbitrarily fixed *in vitro* circumstances. Since the relative activities of sulfonamides are different for different organisms, it is not possible to state that one or another sulfonamide is the more active against all pathogens.[46,47] The differences are seldom great, and whether they are clinically important is difficult to determine from the available data.

Other factors governing the choice of sulfonamides, such as rate of absorption, distribution in body fluids, acetylation, protein binding, excretion rates and over-all toxicity, are complex variables that will not be dealt with in detail in this review. Toxicity has probably been the most important of these considerations to the clinician for, as has been shown in Table III, the clinical results obtained following the use of the various substituted sulfonamides have been similar despite the pharmacologic differences that distinguish these drugs from one another.

Reliable toxicity data are, of course, a function of the care and consistency with which evidence of toxicity is sought. Examining most hospital charts for stated evidence of toxicity will usually yield less evidence of toxicity than regular examination of the patient and the body fluids by individuals particularly interested in the problem. Comparisons of one year with another are not entirely satisfactory unless pains have been taken to insure reproducibility of observation. Not many years ago the urines and bloods of all patients receiving sulfonamides were examined frequently and carefully. As confidence in the less toxic sulfonamides has grown, this practice has diminished to such an extent that the apparent incidence of microscopic hematuria, etc. is undoubtedly lower, from a perusal of hospital charts alone, than it was some years ago. Nissen et al.[48] have studied the problem of renal toxicity with care and have determined that the renal toxicity of several of the commonly used sulfonamides is about the same (about 2.0 per cent incidence of hematuria). They pointed out that an adequate comparison of the renal toxicity of any two sulfonamides would probably require study of at least 1,000 patients under comparable conditions of each drug.

TABLE III
EFFECT OF SULFONAMIDES ON INFECTIONS OF THE URINARY TRACT

Reference	Drug	Organism	Bacteriologic Control (%)
Uncomplicated Infections:*			
34	Sulfanilamide	E. coli	81
34	Sulfanilamide	Staph. aureus	63
35	Sulfanilamide	Mostly E. coli and Staph. aureus	87
34	Sulfapyridine	E. coli	91
34	Sulfapyridine	Staph. aureus	88
29	Sulfapyridine	E. coli	83
29	Sulfapyridine	Staph. aureus	75
34	Sulfathiazole	E. coli	79
34	Sulfathiazole	Staph. aureus	88
36	Sulfathiazole	Staph. aureus	88
37	Sulfathiazole	Mostly E. coli	91
38	Sulfathiazole	Mostly E. coli	81
34	Sulfadiazine	E. coli	88
34	Sulfadiazine	Staph. aureus	91
39	Sulfadiazine	Mostly E. coli	85
40	Sulfamerazine	Bacillary	85
40	Sulfamerazine	Staph. aureus	89
41	Sulfisoxazole	E. coli	87
34	Sulfacetamide	E. coli	93
42	Sulfacetamide	Various	92
43	Sulfadimetine	Sensitive strains	93
44	Sulfadimetine	Various	78
45	Sulfathalidine	E. coli	86
Complicated Infections:†			
35	Sulfanilamide	Various	35
28	Sulfanilamide	Various	58
37	Sulfathiazole	Various	39
38	Sulfathiazole	Various	52
39	Sulfadiazine	Various	17
44	Sulfadimetine	Various	33

* Acute and chronic cases are included; many of these failed to respond to other forms of treatment. The figures do not include prolonged follow-up study.

† Many of these cases are chronic without demonstrable abnormalities of the urinary tract. The studies with the lowest percentage of control almost always included the largest number of follow-up cultures. Probably all of these figures are higher than the actual final rate of control and none should be used to indicate superiority of one drug over another.

Despite the broader *in vitro* range of action of the various sulfonamides, clinical effectiveness is less than the *in vitro* observations indicate in enterococcal, proteus or pseudomonas infections or in mixed infections. This is due to the increased incidence of resistance to sulfonamides in these bacteria (Fig. 1), as well as to the uncommon occurrence of these organisms except in chronic and complicated infections of the urinary tract, in which treatment is less likely to be successful.

Mixtures of sulfonamides, which have a considerable theoretical advantage in terms of solubility in urine, have not received sufficiently detailed clinical study to permit adequate comparisons to be made. There is little doubt of their effectiveness but these mixtures are not entirely free from reactions. The chief objection to their use is that the *in vitro* effects of the combinations are not always predictable and, in some instances, mixtures may be less effective than one of the component drugs alone.[49] The use of mixtures is probably best reserved for specific therapeutic problems in which *in vitro* study may reveal a distinct advantage in their administration.

The choice of a sulfonamide in the treatment of infections has been greatly complicated by conflicting claims, hasty publication and the frequent absence of detailed bacteriologic and clinical study, particularly with respect to prolonged follow-up study. There is still a great

need for comparative study of sulfonamides under rigorous clinical conditions. The exhibition of *in vitro* activity of any drug is a useful guide to therapy only when it has been shown to correlate with clinical response. For example, 2-sulfanilamidopyrimidine (sulfadiazine) is an excellent drug *in vitro* and *in vivo* whereas the 4-sulfanilamido isomer is as effective *in vitro* but ineffective *in vivo*.[50] Sulfisoxazole is relatively active against strains of Proteus *in vitro* but the *in vivo* response has not been so encouraging.[41,51]

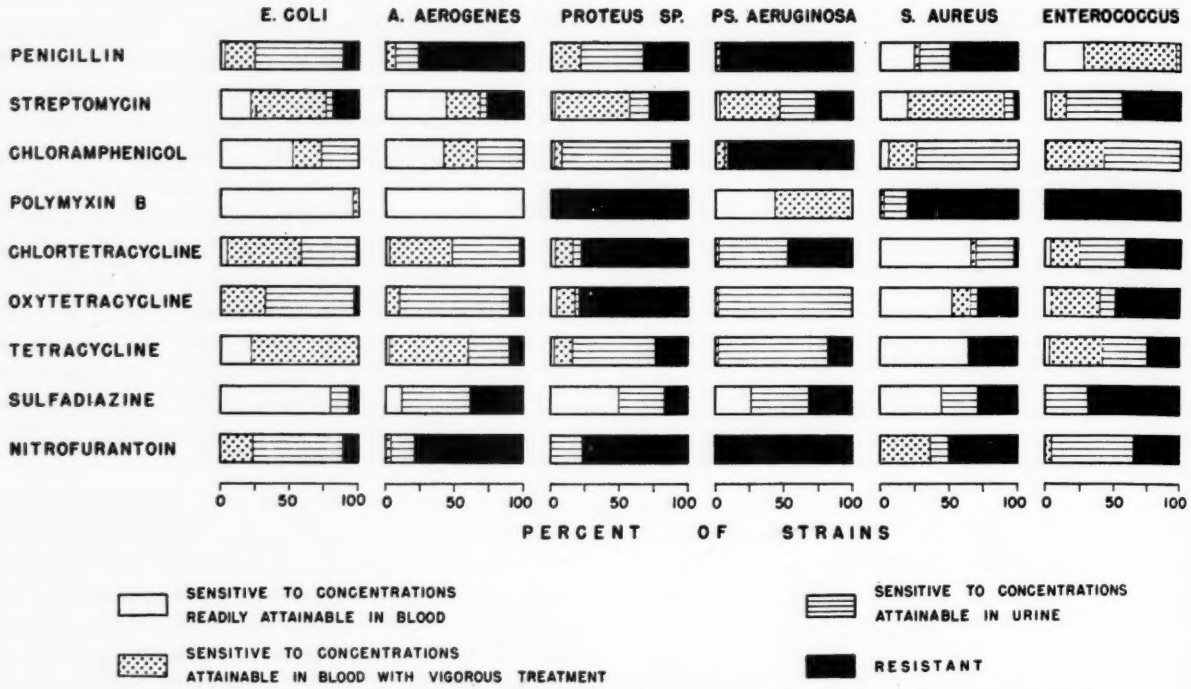

FIG. 1. Sensitivity of common urinary pathogens to nine antibiotic and chemotherapeutic agents.

Many clinical problems in the use of sulfonamides have not received proper study. For example, Sung and Helmholz[52] showed that sulfathiazole was much more active against S. fecalis at pH 5.5 than at pH 7.2, whereas against E. coli the situation was reversed and the drug was markedly less active in the acid range.

At present it would seem wise for the clinician to choose one of the commonly used sulfonamides with a long and clearly proved history of clinical use and effectiveness, and to learn to use it well. Hasty switching to other related compounds should be avoided unless the clinical evidence of the superiority of the alternative is unmistakable.

Antibiotics. With the development of antibiotics the literature on the treatment of infections of the urinary tract changed its emphasis. Studies of antibiotic therapy of infections of the urinary tract naturally began by observations of relatively large numbers of chronic and complicated infections. Although scattered data indicate that the high order of effectiveness of chemotherapeutic agents in the treatment of acute uncomplicated infections is equalled by the newer antibiotics, used properly, precise documentation is meager in terms of careful comparative studies with adequate follow-up. Increasing emphasis must therefore be placed on the effectiveness of a drug in eliminating sensitive bacteria from the urinary tract, even though this may not result in the elimination of all bacteria. In this way an evaluation of the antibacterial effectiveness of an antibiotic *in vivo* may be achieved. However, the common situation in the treatment of chronic and complicated infections is that sensitive bacteria are cleared from the urinary tract and are replaced by resistant bacteria. Unless it can be shown that the resistant strains appear as a result of some direct action of the antibiotic on the bacteria it is probably acceptable to assume that the eradication of sensitive strains is the full limit of effectiveness that can be expected from a given drug. This, as will be seen, is a far cry from the objective of producing ultimate bacteriologic control of the urinary tract infection.

A summary of the range of activity of antibiotic and chemotherapeutic agents *in vitro*

against the six most common pathogens of the urinary tract is presented in Figure 1. The data were collected from many sources but principally from the studies of Finland and his collaborators[15,17,22,53–58] and include unpublished data on nitrofurantoin and on enterococci kindly supplied by Dr. William A. Richards and Dr. Wilfred F. Jones, Jr. The preparation of a figure such as this involves arbitrary judgments and compromises. A certain amount of variation in the data is inevitable, hence small differences among the drugs listed are not necessarily meaningful. Many of the patterns of sensitivity reflect the predominant patterns of antibiotic management in the hospitals from which the strains were collected. The most reliable data for a given hospital would undoubtedly be those collected from its own laboratory, and might vary considerably through the years, presumably as antibiotic practices varied.[58] It is likely that the patterns of sensitivity encountered in office practice would be somewhat different from those presented here.

In choosing the ranges of sensitivity used in Figure 1, arbitrary values were selected as representing four levels of antibiotic. (Table IV.) The first category consists of levels of antibiotic attained readily in the blood after the administration of ordinary doses of antibiotic.

TABLE IV

LEVELS OF ANTIBACTERIAL AGENT ATTAINABLE IN BODY FLUIDS UNDER ORDINARY CONDITIONS

Levels	Penicillin	Streptomycin	Chloramphenicol	Polymyxin B	Chlortetracycline	Oxytetracycline	Tetracycline	Sulfadiazine	Nitrofurantoin
Levels easily attainable in blood* μg./ml.	2	20	3	3	3	3	3	50	0
Levels attainable in blood after vigorous treatment μg./ml.	25	50	12	12	12	12	12	200§	5
Levels attainable in urine μg./ml.	1000	250	200	100	200	200	200	500	10
Resistant μg./ml.	>1000	>250	>200	>200	>200	>200	>200	>500	>10
Ordinary doses gm./day	0.3–0.5†	1.0	2.0	0.10	2.0	2.0	2.0	4.0–6.0	0.3
Vigorous doses gm./day	5.0–8.0†	4.0	4.0 or 1.0‡	0.25	4.0 or 1.0‡	4.0 or 1.0‡	4.0 or 1.0‡	.....	0.6

* Values are approximately those to be expected with properly divided doses in the normal adult. Penicillin, streptomycin and polymyxin B are given intramuscularly and the others orally, unless otherwise indicated.

† Values are given for crystalline penicillin administered intramuscularly. Larger doses are required for repository forms.

‡ Intravenously in divided doses.

§ Ordinarily blood levels of sulfonamide ought not to exceed 150 μg./ml. (15 mg. per cent). In the preparation of Figure 1 insufficient data could be found concerning inhibition of bacteria in the range of 50 to 200 μg./ml. of sulfonamide, hence only the category dealing with levels attainable in urine is charted.

The second category encompasses levels attainable in blood after vigorous treatment. The third category includes levels attained in urine after moderate dosages of drug have been administered, and the fourth represents strains resistant to the latter concentrations of drug. Perhaps the most significant information in Figure 1 is the incidence of resistance to the drugs listed, since this is the least likely value to be affected by the variables involved in the preparation of the data. Each of the drugs charted, and a few others, will be taken up in turn.

Penicillin. Although penicillin has been used very little in the treatment of gram-negative bacillary infections of the urinary tract there were early indications *in vitro* that it might be useful against certain strains of Proteus.[60] No

detailed extension of this observation was made until relatively recently when it was shown that one of the several differences between Proteus mirabilis and the strains of Proteus that produce indole (P. vulgaris, P. morgani and P. rettgeri) is that P. mirabilis is relatively much more sensitive to penicillin than are the latter organisms.[57] The incidence of penicillin sensitivity among strains of Proteus is to a large degree a reflection of the relative proportion of strains of P. mirabilis in the group.

The treatment of staphylococcal infections has become a major problem in the management of infectious disease. The sensitivity of staphylococci to penicillin has decreased steadily as usage of the drug has continued, and the incidence of susceptible strains is now about 25 per cent.[55] However, the penicillin-resistant strains are found largely in hospitals, and the data derived from careful bacteriophage typing of staphylococci obtained from hospital personnel as well as from infected patients indicate that the resistant staphylococci are largely nosocomial in origin.[61] Patients with no history of recent hospitalization or recent administration of penicillin usually harbor penicillin-sensitive staphylococci. Thus penicillin is likely to be effective in staphylococcal infections of the urinary tract in patients with no recent hospitalization, instrumentation or penicillin therapy.

Penicillin has been effective in clearing many enterococcal infections of the urinary tract.[17,43] Enterococcal infections usually are a consequence of instrumental manipulation of the urinary tract and are much more common in females than in males.[62] When these organisms occur in urinary tracts it is usually in association with other bacteria. These factors, and the usual association of instrumentation and mixed cultures with chronic and complicated infections, indicate that bacteriologic control of these infections is often difficult.

One of the difficulties that may be encountered in the treatment of enterococcal infections of the urinary tract with penicillin is the peculiar property of some enterococci of being killed more rapidly by optimal concentrations of penicillin than if the optimal concentration is greatly exceeded.[63] Too much penicillin may interfere with the therapeutic effect under such circumstances. Penicillin is, fortunately, one of the least toxic of the commonly used drugs. The extraordinary dosage range over which it can be administered without significant reactions may perhaps have led to failure to appreciate the limitations of the dose ranges of most other antibacterial drugs. The manifestations of sensitivity to penicillin* have been reviewed by Finland and Weinstein.[64] The incidence of untoward reactions following the use of repository forms of penicillin is significantly greater than that due to crystalline penicillin alone.

Streptomycin. The effect of streptomycin on urinary tract infections has been extensively studied and there is good agreement between clinical results and *in vitro* observations, if the pH of the urine is maintained at proper levels.[65–67] Streptomycin activity increases five- to tenfold with each unit increase in pH, hence the administration of alkali with streptomycin is important. Alkaline urines may occur in proteus infections, as a result of urea splitting, and it is occasionally suggested that the use of additional alkali is unnecessary under such circumstances. However, as soon as bacterial production of urease has been inhibited, as a result of the action of streptomycin, the pH of the urine may fall promptly. This causes a rapid decrease in the effectiveness of the streptomycin just when the highest activity of the antibiotic is needed to eliminate the last surviving pathogens in the urine. Hence, alkali (as sodium bicarbonate or alkaline citrates) should be administered throughout the course of the infection regardless of the effect of urease-producing bacteria, and the urine tested frequently, through the use of indicator papers, to determine that the pH has remained as near 8.0 as possible.

The capacity of streptomycin to induce drug resistance is probably greater than that of any other commonly used antibiotic and probably accounts for many treatment failures with this drug. Because the emergence of resistance may be so complete and because streptomycin, when it is effective, usually kills bacteria so rapidly, treatment with this drug ought not to be prolonged more than four to five days except under unusual circumstances.

Bacteriologic control of acute infections of the urinary tract is about 60 to 80 per cent effective with streptomycin.[65] These figures are lower than those obtained with other drugs; this is due, in part, to the failure to use alkali in some of the earlier observations, and probably also to the ready emergence of resistance to this antibiotic.

* See also the paper by Von Oettingen. *Am. J. Med.*, 18: 792, 1955.

Bacteriologic control in chronic infections of the urinary tract has varied between 17 to 87 per cent; the latter figure would probably be reduced by more extensive follow-up study.[65]

The activity of streptomycin against staphylococci is greater than is usually thought and should not be overlooked. Streptomycin has been particularly useful in the management of staphylococcal and enterococcal infections in combinations with other effective drugs.

Occasionally streptomycin-dependent organisms may emerge during treatment—these disappear when streptomycin is no longer available to them.[68]

The vestibular toxicity of streptomycin is well known, and streptomycin may also induce severe epidermal sensitization reactions, as well as other evidence of toxicity. Although dihydrostreptomycin is less likely than streptomycin to produce vestibular damage, it is more likely to produce severe deafness. The latter may be a late and irreversible complication.[59] For this reason dihydrostreptomycin is probably best reserved for patients who have given evidence of toxicity to streptomycin.

Chloramphenicol. The first broad-spectrum antibiotic to be reported in the literature, chloramphenicol, has demonstrated its value in the treatment of urinary tract infections. Among its noteworthy properties are ease of administration and relative freedom from side effects, but dizziness and mucocutaneous lesions not infrequently occur during its use.[59] The appearance of serious and even fatal hematologic complications has been sufficiently infrequent that the drug ought not to be withheld in cases of serious infection in which it may be useful, if careful observation of the patient can be accomplished.

However, there are several other properties of chloramphenicol that complicate its use in urinary tract disorders. The excretion pattern of the active, unconjugated drug is variable and unpredictable.[59] Chloramphenicol is least active against E. coli at pH 6.6 and is more active on either the alkaline or acid side of this value *in vitro*. The incidence of frankly resistant strains of E. coli, A. aerogenes, Proteus, enterococcus or staphylococcus is low but the incidence of highly susceptible strains is also low. Thus chloramphenicol should be given in fairly large doses (2 to 4 gm. per day). The drug is virtually useless against Ps. aeruginosa. The variable effects of antibiotics in inducing drug resistance are illustrated by the observation that staphylococci exposed to chloramphenicol develop resistance relatively slowly[69] whereas E. coli, under similar conditions, may become resistant more rapidly.[70]

The clinical evaluation of chloramphenicol has shown excellent agreement between *in vitro* sensitivity and elimination of organisms from urinary tracts.[71] However, here, as with other drugs, the incidence of ultimate bacteriologic control is much related to chronicity of infection and the presence of abnormalities in the urinary tract and to antibiotic sensitivity.[71,72]

Polymyxin B. The high order of effectiveness of polymyxin B against E. coli, A. aerogenes and Ps. aeruginosa is well known, but because of the high incidence of renal and neurologic toxicity associated with the use of the drug and the availability of alternative therapy, polymyxin B has not been widely used for treating any but resistant and complicated infections. However, the renal toxicity of polymyxin B is almost always reversible.[73–75] Other toxic manifestations have also included local pain, somnolence, nausea, vomiting, anorexia and dizziness but these have seldom been serious. This drug merits wider study than it has received.

Polymyxin B induces resistance in susceptible organisms very slowly[76] *in vitro*, but resistant bacteria may appear promptly in complicated urinary tract infections that are refractory to therapy. This suggests that reinfection with resistant strains rather than mutation of organisms already in the urinary tract may be the more likely mechanism of emergence of organisms resistant to this drug.

Chlortetracycline, Oxytetracycline and Tetracycline. Chlortetracycline is probably more active *in vivo* than is indicated by the *in vitro* observations since 50 to 75 per cent of the drug in culture media deteriorates during the testing period. It is probable, if suitable correction is made for this *in vitro* deterioration, that chlortetracycline is the most active of the tetracycline group of antibiotics against most susceptible bacteria. The relatively great effectiveness of all three of these drugs and their ease of administration has led to their widespread use in infections of the urinary tract. A close correlation (90 to 95 per cent) has been observed between *in vitro* sensitivity and clearing of the organisms from urinary tracts.[77–79] However, failures frequently due to reinfection with different organisms have occurred in chronic and complicated infections,

and the relapse rate after apparent control of infections of this type has been high.

The tetracycline group is especially active against E. coli, A. aerogenes, staphylococci and enterococci. Oxytetracycline has greater effectiveness than its congeners against strains of Ps. aeruginosa. (Fig. 1.)

The differences between P. mirabilis and the indole-producing strains of Proteus with respect to sensitivity to penicillin have been commented upon.[57] Whereas P. mirabilis is more sensitive to penicillin than are the indole-producing strains, the latter are much more sensitive to the three tetracyclines. As strains of P. mirabilis decrease in incidence in any hospital the tetracyclines may increase their effectiveness against proteus infections because of this difference. Tetracycline is more effective than its congeners in inhibiting strains of P. mirabilis.

Resistance of staphylococci to tetracyclines has increased progressively.[55] Whereas staphylococci were originally almost all susceptible to the tetracyclines, resistance now occurs in about one-third of strains, undoubtedly as a result of the widespread use of these drugs. It is noteworthy that chlortetracycline, although the first of these three drugs to be introduced, has maintained a small advantage over its two congeners in terms of *in vitro* antistaphylococcal activity, and this despite the greater lability of chlortetracycline during the testing procedure.

The major difficulties that have arisen in the use of the tetracyclines have been their tendency to induce gastrointestinal and mucocutaneous disturbances. The exact incidence of such disturbances varies with the three analogues, the type of patient, the intensity of observation, dosage and other variables. A controlled study in a large municipal hospital showed the incidence of such disturbances to be more frequent following the administration of oxytetracycline than following chlortetracycline.[80] Tetracycline was least likely to induce these difficulties but the experience with the latter drug has been relatively brief. Staphylococcal diarrheas, one of the most serious complications of antibiotic therapy, were significantly less frequently encountered in those treated with chlortetracycline than after the use of oxytetracycline,[80] and were still less frequent after treatment with tetracycline.[79]

Although the three tetracycline drugs usually act similarly on given bacterial strains, significant differences may appear at times, as has been pointed out. It is therefore recommended that sensitivity to each of these drugs be determined separately whenever a difficult therapeutic problem is encountered.

Nitrofurantoin. This drug is not an antibiotic but has been used recently in the treatment of infections of the urinary tract. Although published experience is small, it is already clear that nitrofurantoin is effective in clearing 75 to 80 per cent of acute infections of the urinary tract, but only a small percentage of chronic complicated infections.[81] It is noteworthy that therapeutic amounts of this drug give undetectable or extremely low blood levels, hence the activity of the drug at "tissue" levels is necessarily limited. About one-fourth of patients receiving therapeutic amounts of the drug complain of gastrointestinal disorders.[81] The structural formula also suggests that hematologic and perhaps other side effects may occur.

Erythromycin. This drug has an antibacterial spectrum similar to that of penicillin. Its major use in the treatment of urinary tract infections is in relation to staphylococcal or enterococcal infections. Resistance of staphylococci to erythromycin may emerge with extreme rapidity *in vitro* and *in vivo*, despite the initial susceptibility of most staphylococci to this drug.[82] Combinations of erythromycin with other drugs, particularly with chloramphenicol or streptomycin, delay the emergence of drug resistance *in vitro*[69] provided that the organisms are initially sensitive to both agents. Toxicity is uncommon and largely limited to the gastrointestinal tract.[82]

Carbomycin. This has been demonstrated to be less effective than erythromycin and to have a similar range of activity. Although the drug may have some value in the treatment of enterococcal infections of the urinary tract,[83] there seem to be no advantages over the use of the more active and probably related drug, erythromycin.

Bacitracin. This is ineffective against all of the common pathogens of the urinary tract except for staphylococci. Its renal toxicity is such that the use of the drug for the treatment of staphylococcal infections should be limited to instances in which other drugs are ineffective.[84]

Neomycin. This is highly effective *in vitro* against E. coli, A. aerogenes, Ps. aeruginosa and S. aureus. Because of its nephrotoxicity and severe ototoxicity when used parenterally the drug can be recommended for urinary tract infections only in desperate situations.[85]

If sensitivity and resistance to antibacterial drugs are determined at levels approximating

those shown in Table IV, a high correlation is found between *in vitro* and *in vivo* results in infections of the urinary tract. Giertz and Gullbring[43] characterized 108 strains of urinary pathogens as "sensitive" to sulfadimetine, penicillin or streptomycin, and found that 95 per cent of these strains disappeared during treatment with the appropriate drug. Conversely, 180 strains were "resistant" and only 4 per cent of these disappeared as a consequence of treatment. Ten strains were intermediate in sensitivity and half of these disappeared during treatment. Most of the patients in this study had chronic or complicated infections.

Similar information was obtained by Högman and Tillegård who showed that 91 per cent of bacteria sensitive to sulfonamides, penicillin, streptomycin, chlortetracycline, oxytetracycline or chloramphenicol were eliminated, and only 6 per cent of the resistant strains were eliminated from the urinary tract. Interestingly, correlations for chlortetracycline and oxytetracycline were 97 per cent but those for chloramphenicol were only 54 per cent—many sensitive bacteria failed to respond to chloramphenicol *in vivo*.

However, the clinical response to chemotherapy and antibiotics has not been as favorable as these data would indicate. It has been repeatedly stressed that the control rate in chronic and uncomplicated infections is lower than in acute, uncomplicated infections. Garrod et al.[62] studied over 1,000 cultures derived from 686 patients who had been treated with sulfonamides or a wide variety of antibiotics. Immediate control of the urinary tract infection was achieved in 89 per cent of those patients with acute uncomplicated infection and sensitive single cultures of organisms, in 83 per cent of those with susceptible mixed cultures, and even in 8 of 10 patients with resistant bacteria but uncomplicated infection. The patients with chronic uncomplicated infections showed a 75 per cent response to treatment when infected with sensitive organisms, 52 per cent when infected with resistant organisms, and there was 44 per cent sterilization of the urine when multiple organisms were present. Those with demonstrable urinary tract abnormalities did not fare so well. Only 47 per cent of males and 61 per cent of females infected with sensitive bacteria developed sterile urines, and 17 per cent of patients with resistant organisms became free of apparent infection. In addition, the relapse rate was exceedingly high. During the few months after discharge about 11 per cent of patients with acute uncomplicated infections, 36 per cent of those with chronic uncomplicated infections and 74 per cent of those with chronic complicated infections relapsed bacteriologically. Similar data have been obtained by Rhoads et al.[3] who found that only 8 per cent of their patients with chronic obstructive disease of the urinary tract were rid of their infections upon prolonged follow-up study.

Thus, although there is a high likelihood that susceptible bacteria will be cleared from the urinary tract by proper antibacterial measures, clinical control of the infection is dependent largely upon the nature of the urinary tract lesion. Three possible explanations may be offered for this paradoxic situation. First, antibacterial therapy may have failed to achieve complete eradication of the pathogen from the urinary tract so that at some later time the original infecting organism may emerge again and cause infection. Such a situation would be likely in chronically infected kidneys, where large scarred areas, obstructed tubules and similar local abnormalities might create circumstances in which bacteria might remain in relative quiescence, and in which only high blood levels of drug, which would permit penetration into such areas, might be expected to be of any value. Such circumstances may account for the emergence, after apparent sterilization of the urine, of the same species of organism with the same antibiotic sensitivity as the original infecting organism. This situation has been reported for about half of the chloramphenicol-treated patients with chronic and complicated urinary infections in one series.[46] To what degree such a mechanism accounts for relapses and treatment failures in acute uncomplicated pyelonephritis (these may number up to 25 per cent of the total treated group)[87] is difficult to determine from available data.

Second, antibacterial therapy may have led directly to the emergence of drug-resistant strains. It is conceivable that in chronic and complicated infections only small amounts of drug may penetrate into a given relatively avascular locus of infection, and this concentration of drug be insufficient to produce eradication of all the bacteria but sufficient to induce the emergence of resistant mutants. It is generally agreed, also, that the larger the number of bacteria to be killed by a given concentration of drug, the less efficient that concentration of

drug will be. The effectiveness of a given drug in heavily overgrown urine may be seriously impaired, thus paving the way for survival of some organisms and emergence of resistant variants. Such a mechanism probably is important in therapy with streptomycin, and may be important in treatment with other drugs, although the magnitude of the problem is not yet clearly defined.

An approach to therapy on this basis lies in the use of multiple drugs. Although combinations of drugs occasionally are mutually inhibitory, they are more commonly additive or synergistic.[88] The rate of emergence of resistance *in vitro* is distinctly less when an effective combination of drugs is used than when either drug is used alone.[69] However, the effectiveness of given combinations is not predictable in all instances and insufficient study has been devoted to the effect of such combinations on pathogens of the urinary tract. When combinations of drugs have been used they have not been remarkably successful. Thus Kimmelman et al.[89] treated thirty-seven patients with a combination of sulfonamide, penicillin and streptomycin. Fourteen of sixteen uncomplicated cases were freed of bacteria and all of the remaining patients were treatment failures—nineteen of the twenty-one failures had anatomic abnormalities of the urinary tract. The net effect was similar to that obtained with single drugs, with the exception that the organisms obtained at the end of the treatment period were uniformly resistant to all three drugs rather than to any one. Some advantage to drug combinations has been claimed by others, although the advantage is not great.[3] Observations of this sort do not prove that emergence of drug resistance in the original infecting strain is not a factor in treatment failures but do indicate that other mechanisms may be operating. Precise study is needed of the importance of variation in resistance *in vivo* particularly in relation to the newer antibiotics.

Finally, the poor results obtained in chronic and complicated infections may be explained as being due to reinfection with organisms different from those originally present. Such organisms would virtually always be resistant to the drug that was previously used. Garrod et al.[62] found that about half of the 148 patients who were treatment failures and whose initial cultures showed only a single bacterial species became reinfected with different organisms during or shortly after treatment. Similar observations are the common experience.[22,43,71,78,83]

The precise incidence of reinfection with bacterial strains different from those originally present is manifestly a function of the detail with which the search for strain differences is carried on. Evidence obtained from study of bacteriophage typing of staphylococci[61] and serologic typing of organisms of the Klebsiella-Aerobacter group[90] shows clearly that strain differences may appear within a species and may be unrecognizable by common clinical bacteriologic methods. The study of such strain differences has demonstrated that there tend to be characteristic ecologic patterns of bacteria within hospitals. Øskov[90] studied 400 urinary strains of the Klebsiella-Aerobacter group. The type-distribution of the strains on male surgical wards was remarkably uniform, that on the medical wards much less so. The most likely explanation of these findings was that there was significant cross infection on the male surgical wards, presumed to be through the medium of instrumentation and catheterization. Similar observations have been made with respect to hospital cross infections with staphylococci.[91]

Such findings suggest that most reinfections may be due to different strains of bacteria from those originally present, even though the species may be the same, but do not explain why reinfections are most common in patients with chronic, complicated infections of the urinary tract.

Halkier and his co-workers[92] studied the relationship of urinary tract obstruction to the frequency of sterilization of the urine and found that the initial course of therapy in patients with prostatic enlargement resulted in 15 per cent bacteriologic control, and only 17 per cent of the patients were freed of infection after prostatectomy. The patients with obstruction due to ureteral stone responded to initial therapy in 63 per cent of cases and 89 per cent were freed of infection after removal of the stone. The factor that seemed most likely to explain the different results in the two groups of patients was that catheterization and instrumentation were frequently employed in the group with prostatic disease, both before and after operation, but such procedures were infrequent in patients with ureteral stone.

Jönsson and Erlanson[22] studied 579 patients with urinary tract complications. Half of these patients were operated upon and 180 of the 290

were infected on admission. Although 40 per cent of the infected patients were treated before surgery, almost two-thirds of them were still infected at the time of surgery. The patients who were uninfected at the time of admission numbered 110, and twenty-six of these became infected while in the hospital—almost all of the twenty-six were patients with prostatic or chronic vesicular disease. Despite surgery and vigorous antibacterial therapy about 60 per cent of the patients who underwent surgery were still infected at the time of discharge, and the incidence of infections in patients with obstruction in the lower urinary tract was three times as great as in those with ureteral obstruction. Prophylactic therapy was used in many of these patients with no apparent benefit. The authors stress that repeated catheterizations, inlying catheters and similar instrumentations were the major differences in the management of the two groups of patients.

Similar data have been derived for S. fecalis infections by Garrod et al.[62] who showed that almost 20 per cent of women who had been catheterized within the previous month developed S. fecalis infection, but only 2 per cent of those who had not been catheterized and returned for follow-up study became infected with this organism. Furthermore, the patients who had received sulfonamides at the time of catheterization had about twice the incidence of S. fecalis infection as those who had not been given sulfonamides at the time of catheterization.

The ineffectiveness of prophylactic drug during catheterization was also shown by Blahey.[93] If oxytetracycline were given prophylactically to female patients undergoing corrective genital tract surgery (the use of inlying catheters is almost universal in such instances), the pyuria was reduced to less than half, as compared with untreated controls, but bacilluria was not significantly reduced. Instead of a predominance of E. coli, as occurred in the control group, there was a predominance of Proteus and Staphylococcus in the urine of the patients who were given prophylactic drug. There was no significant net benefit in terms of bacilluria but the bacteria had been altered from those easily controlled by therapeutic means to those that are more troublesome.

There seems little doubt, at present, that nosocomial infections play a large role in the pathogenesis of many urinary tract infections, and that catheterization and other instrumentation are the major carriers.[94] Prophylaxis may offer a false sense of security by temporarily reducing pyuria but serves largely to replace susceptible with resistant organisms without reducing the actual rate of infection.

The status of treatment in chronic and complicated infections of the urinary tract may be summarized briefly:

1. Eradication of drug-sensitive bacteria is possible more than 90 per cent of the time.

2. Reinfection with drug-resistant bacteria occurs so frequently after the urine has become sterile that the permanent control rate in these infections is probably no greater than about 10 per cent.[3,62,95]

3. When reinfection occurs it is most often due to bacterial strains different from those originally present, although it may be due to the same species.

4. Nosocomial infections play a large role in the reinfection of urinary tracts, probably through the medium of the catheter and similar instruments.

5. Prophylactic therapy in association with catheterization and instrumentation may create new bacteriologic problems.

Among the unsettled problems is the question of the source of infection in the chronic infections that are not associated with instrumentation, catheterization or obvious abnormality of the urinary tract.[96] Suggestions concerning the source of such organisms have been many, but are unproved. A clear evaluation of the benefits and risks of instrumentation and catheterization is needed. It is almost inevitable in this era that patients with recurrent infections of the urinary tract be subjected to detailed retrograde study. In view of the generally depressing situation with respect to infections of the urinary tract, a more precise evaluation of the needs and indications for such study may be of benefit.

There can be little doubt that the use of inlying catheters is much abused in current hospital practice, yet the procedure is clearly unavoidable at times. If it be assumed that entry of new bacteria to the urinary tract, in such instances, is along the urethra, it becomes conceivable that the utilization of antibacterial solutions in the bladder to provide bacteriostasis and drainage of the organisms so introduced has merit. The use of tidal drainage[97] and its modifications has proved its efficacy in reducing the incidence of fatal infections of the urinary tract in patients with neurologic disturbances in

whom constant urinary drainage through catheters is required. More widespread use of this and similar procedures on surgical, urologic and medical wards is indicated.

Non-specific means for reducing the bacterial count in urine may have advantages other than prophylactic. On several occasions we have observed that when bacterial counts were reduced below about one million bacteria per ml. of urine symptoms of dysuria and frequency diminished in intensity, even though bacteria were demonstrable in the urinary tract. This may account for the greater frequency of symptomatic relief than of bacteriologic sterility in infections of the urinary tract. It also suggests that reduction of the bacterial count by non-specific measures may provide symptomatic relief in otherwise intractable infections. This principle has been applied successfully in selected patients with chronic infections of the urinary tract, and symptomatic relief has occurred frequently when the bacterial count was lowered by tidal drainage, urinary antiseptics, prolonged treatment with small doses of antibiotic or similar procedures.

The over-all impact of recent advances in therapy of urinary tract infections is difficult to assess. Although a distinct reduction in the incidence of pyelonephritis at autopsy has been reported from one hospital,[4] it is difficult to state that this is due to improvements in therapy. The same study indicates that pyelonephritis was diagnosed at autopsy about five times as often as the clinical diagnosis was made, hence treatment was unlikely to be the deciding factor. It is noteworthy that Loopuyt[98] found that the incidence of clinically asymptomatic infections was significantly greater among patients on the public wards than among patients in the private sections of the hospital in which he worked. The significance of this observation is not yet clear but it suggests that non-specific factors may be operating to cause changes in the incidence of pyelonephritis in certain population groups. Such factors as intensity of medical care, hygienic habits, maternal and obstetrical care, predisposing diseases, and many others may be implicated in accounting for the difference between the two economic-social groups but there are insufficient data to support any of these conjectures.

SUMMARY AND CONCLUSIONS

1. Infections of the urinary tract are among the most common clinical infections and the late sequelae are clinically significant in relation to diabetes, pregnancy, malignant hypertension, uremia and probably other disease states.

2. Pyelonephritis is frequently asymptomatic, usually causes symptoms referable to the lower rather than the upper urinary tract, and is diagnosed clinically in only about one-fifth of the cases in which it is found at autopsy.

3. Bacilluria is the most significant finding and significant bacilluria occurs when more than 10^5 bacteria per ml. are found in the urine. Certain defined circumstances which inhibit bacterial multiplication in urine may lead to lower bacterial counts in urine in the presence of infection. The Gram stain is a useful clinical tool to distinguish urines with high bacterial counts from those with low bacterial counts. Most of the latter are due to contamination although there are specific exceptions that are discussed in detail. Cultural data without Gram stain or quantitative count of the urine are difficult to interpret because of the high incidence of contamination in the collection of specimens of urine.

4. Although E. coli and Staph. aureus are the most common urinary pathogens, strains of Proteus, Pseudomonas and enterococcus are more frequent in chronic and anatomically complicated infections than in uncomplicated infections. Enterococci are commonly encountered in women as a consequence of catheterization. Cultures containing more than one bacterial species are usually indicative of chronic or complicated infections.

5. A brief review of older methods of treatment of urinary infections shows that ketone bodies, mandelic acid and methenamine mandelate, when used properly, are highly effective in the treatment of most acute uncomplicated infections of the urinary tract but relatively ineffective in the treatment of chronic and complicated infections.

6. The sulfonamides show the same pattern of response in relation to chronicity and anatomic lesions, but are probably slightly more effective than the methods of treatment already mentioned. Very little basis exists for choosing one or another of the commonly used sulfonamides, and the problems of toxicity, relative activity and basis for choice, on clinical grounds, are reviewed to show that the various pharmacologic differences among the sulfonamides are seldom reflected in altered incidence of clinical and bacteriologic control of infections.

7. The present status of resistance and susceptibility of the six most frequently encountered urinary pathogens to the commonly available antibiotic and chemotherapeutic agents is presented. The advantages and disadvantages of each antibiotic are discussed briefly and their clinical effectiveness is assessed.

8. Under proper conditions correlation is better than 90 per cent between antibiotic sensitivity and elimination of bacteria from the urine. However, in chronic, complicated infections, reinfection and relapse rates are so high that ultimate bacteriologic control probably does not exceed 10 per cent.

9. Reinfection is usually nosocomial in origin in chronic urinary tract infections, and instrumentation and catheterization are the major sources of the new infections. Certain strains of bacteria become prevalent on hospital wards and become the predominant ones where inlying catheters, etc. are in widespread use.

10. Prophylactic use of drugs in patients with inlying catheters is probably valueless and serves merely to convert the bacteriologic flora from susceptible to resistant to the prophylactic agent.

11. Multiple-drug therapy would be expected to be of value if the appearance of resistant strains of bacteria in unsuccessfully treated cases were due to emergence of resistant variants of the original strains. Such emergence probably occurs with drugs such as streptomycin but is undoubtedly less common than introduction of new organisms of nosocomial origin. A few clinical trials with multiple drugs have shown little advantage to their use, and the disadvantage of resistant to all the drugs in the combination emerges. In properly selected cases, however, combinations may still be of value.

12. Although rational use of chemotherapeutic and antibiotic agents offers much to patients with infections of the urinary tract, our present state of knowledge is so incomplete that a high percentage of failure in certain types of infection will characterize therapeutic activity until more fundamental knowledge concerning infections of the urinary tract has been accumulated.

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The Chemotherapy of Bacterial Infections Refractory to the Common Antibiotics*

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In the last decade the clinical picture of infectious disease has undergone a rapid and radical change notable for the decline of such virulent bacteria as the pneumococcus and beta hemolytic streptococcus to positions of minor clinical importance and the rise of organisms formerly considered semi-saprophytes and minor pathogens as serious therapeutic problems. These semi-saprophytic organisms include Pseudomonas aeruginosa, the coli-aerogenes group, Bacillus proteus, the enterococcus and Staphylococcus aureus, all of which grow well both in and free of the animal body and carry out their life cycle in the simplest media. They are found with increasing frequency to be the cause of infections that are resistant to the chemotherapeutic agents in general use. It would appear that the organisms perhaps lower in the process of evolution and, therefore less specialized as pathogens, have, with their ability to synthesize the most complex molecules from the simplest ones, greater adaptability under the adverse conditions imposed by antibiotic therapy. This might be made possible by their being in possession of many alternative metabolic pathways.

It is these five groups of bacteria including the staphylococcus, the enterococcus, B. proteus, Ps. aeruginosa and the coli-aerogenes group, the infections they cause and the chemotherapeutic agents utilized in their treatment that we will consider. (Table 1.) Infections produced by these organisms are becoming more prevalent as their more virulent bacterial competitors are eliminated by chemotherapy.

Refractory infections based on the presence of foreign bodies or deficient host resistance factors such as those associated with calculi, urinary tract obstruction, packings, drains, catheters, orthopedic appliances, agranulocytosis, lymphomas, leukemias, metastatic carcinomas, chronic scar tissue, necrotic tissue, fistulas and undrained collections of pus can only occasionally be successfully treated with chemotherapy of any type. The need for proper surgical therapy such as aseptic technic, the removal of foreign bodies, devitalized tissue, fistulas or feeding avenues of infection, obstructions and the drainage of purulent collections is in no way supplanted by antibiotic therapy. Likewise, there is no good evidence that chemotherapy will more than indirectly aid the healing of tissue already damaged by reducing further destruction. Nor will such therapy neutralize the toxins already formed. The accuracy of the diagnosis as well as the local and systemic host factors must receive first consideration in the successful attack on refractory infections.

REFRACTORY BACTERIAL INFECTIONS

B. proteus: This pleomorphic, motile, gram-negative, ammonia-producing bacillus is the frequent cause of extremely resistant genito-urinary infections and occasionally produces fulminating systemic sepsis. Although it has been found that this organism is most actively killed or inhibited by neomycin[1–5,79] in extremely low concentration, the toxicity of this agent, which will be considered later, has limited its general use. However, chloramphenicol (chloromycetin®), sulfonamides or furadantin® may be used effectively in combinations or occasionally singly against urinary tract infections with this organism. Streptomycin is frequently active against B. proteus but, as in most other infections, it rapidly selects out the resistant strains of this bacillus and should always be used in combination with other chemotherapeutic agents. In this way the emergence of resistant organisms may be delayed or prevented. However, when the organism is resistant to these chemotherapeutic agents, neomycin® may be used under close

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observation, sometimes with dramatic and even life-saving effect.

Neomycin, like streptomycin, bacitracin and polymyxin, is poorly absorbed, if at all, from the intestine. For systemic action these antibiotics must therefore be administered intramuscularly since they are much too toxic when given intravenously. As is noted with polymyxin and bacitracin, neomycin may produce renal tubular damage although it is less toxic than these antibiotics in this respect. In addition, as is observed with dihydrostreptomycin, it may cause deafness.[5] However, these reactions can be minimized or avoided with proper dosage and observation.[58] Patients with septicemias, endocarditis and abscesses, as well as those suffering from urinary tract infections, have been successfully treated with this antibiotic. The bactericidal[1] action of neomycin makes it particularly helpful in the treatment of endocarditis

TABLE I

POTENTIALLY REFRACTORY BACTERIA AND THEIR CHEMOTHERAPY

Organism	Infections	Chemotherapeutic Agents	Dosage	Activity	Toxicity
B. proteus	Genitourinary tract, skin, burns, wounds, septicemia, abscesses, meningitis, endocarditis	Chloramphenicol	P.O., 40–80 mg./kg./day; I.M. or I.V., 10 mg./kg. every 8–12 hr.	Bacteriostatic	Blood dyscrasia, diarrhea, nausea, vomiting
		Sulfonamides	P.O., 40–120 mg./kg./day	Bacteriostatic	Hypersensitivity, renal
		Furadantin (for urinary tract infections only)	P.O., 5–10 mg./kg./day	Bacteriostatic	Diarrhea, nausea, vomiting
		Streptomycin (to be used in antibiotic combinations only)	I.M., 20–40 mg./kg./day	Bactericidal	Eighth nerve, hypersensitivity
		Neomycin	I.M., 10 mg. (2000 units)/kg./day	Bactericidal	Eighth nerve, renal
Ps. aeroginosa	Genitourinary tract, skin, burns, wounds, septicemia, meningitis, endocarditis	Oxytetracycline	I.V., 10 mg./kg. every 8–12 hr., P.O., 30–60 mg./day	Bacteriostatic	Diarrhea, nausea, vomiting
		Chloramphenicol, sulfonamides, furadantin, streptomycin	As above	As above	As above
		Polymyxin	I.M., 2–5 mg./kg./day (each dose 1 mg./kg. or less)	Bactericidal	Renal, fever, neurologic (paresthesias, hypesthesia, ataxia), hypersensitivity
Coli-aerogenes	Genitourinary tract, biliary system, peritoneum, septicemia, meningitis, endocarditis	The tetracycline antibiotics,* chloramphenicol, sulfonamides, streptomycin, polymyxin, neomycin	As above	As above	As above
Staph. aureus	Skin, mucous membranes, pneumonia, genitourinary tract, abscesses, septicemia, osteomyelitis, meningitis, endocarditis	Penicillin (to be used in combinations)	I.M. or I.V., 1–10 million units/day	Bactericidal	Hypersensitivity
		Erythromycin	P.O., 20–60 mg./kg./day; I.V., 10 mg./kg. every 8–12 hr.	Bacteriostatic or bactericidal	Nausea, vomiting, diarrhea
		Chloramphenical, tetracycline antibiotics,* streptomycin, sulfonamides, neomycin	As above	As above	As above
		Bacitracin	I.M., 1,000 units/kg./day (divided in doses every 6 hr.)	Bactericidal	Renal
Enterococcus	Genitourinary, gastrointestinal and respiratory tracts, septicemia, endocarditis	Penicillin, streptomycin, erythromycin, chloramphenicol, tetracycline antibiotics,* sulfonamides, bacitracin	As above	As above	As above

* Tetracycline antibiotics: chlortetracycline, oxytetracycline and tetracycline.

due to refractory organisms.[51,66,77] Resistance to neomycin of clinical significance has been uncommon.

Ps. aeruginosa: This group of gram-negative, motile bacilli characteristically produce a bluish-green, water-soluble pigment which is itself antibacterial for some organisms. Since the clinical use of antibiotics has become so widespread, this organism is found with increasing frequency as the cause of infections of the ear, skin, burns, urinary tract, meninges, blood and heart valves. These infections on occasion may respond to therapy with streptomycin (when used in combination with other agents), oxytetracycline (terramycin®), chloramphenicol (chloromycetin), sulfonamides or furadantin. However, when such agents are unsuccessful or a septicemia, meningitis or bacterial endocarditis is caused by this organism, polymyxin (aerosporin®) has proved most active *in vitro* and is clinically effective.[6–8,36,37,80–94,111–114] Neomycin is a good second choice and has similar activity.[2,3]

Polymyxin, as noted previously, is poorly absorbed from the gastrointestinal tract and is usually administered intramuscularly. This may be done with safety if the proper dosage is given and all renal factors are evaluated. Like neomycin, it is bactericidal in its activity upon microorganisms. Resistance to polymyxin is difficult to produce *in vitro*[8] and is rarely noted clinically.

The coli-aerogenes group: These gram-negative bacilli, normal inhabitants of the gastrointestinal tract of man and animals, are being found more and more frequently as the cause of refractory infections of the genitourinary tract, gallbladder, biliary system, peritoneum and blood. The Aerobacter aerogenes has been a particularly disturbing problem in that resistant mutants tend to be selected out on exposure to antibiotics.[9,42,58] This has been noted in attempts at local sterilization of the intestinal tract with neomycin prior to surgery on the large intestine.[42] Although the majority of coli-aerogenes organisms still are within the therapeutic range of chlortetracycline (aureomycin), tetracycline (achromycin,® panmycin, steclin, tetracyn, etc.), oxytetracycline (terramycin), chloramphenicol (chloromycetin), sulfonamides or streptomycin (when used in combination with other chemotherapeutic agents), there has been a gradual but steadily increasing resistance to many of these antimicrobial durgs.[9] When such resistance to these agents is encountered, polymyxin or neomycin may be successfully employed if proper precautions are observed.

Staph. aureus: This organism is a common parasite on the skin and mucous membranes but will invade the blood stream and produce septicemia, pneumonia, genitourinary infections, bacterial endocarditis, meningitis, osteomyelitis and suppurative lesions anywhere in the body. It also causes the most frequent type of food poisoning by producing a powerful enterotoxin. Most of the virulent strains are coagulase-positive and produce hemolysis on blood agar.

This organism has evinced a progressively increasing resistance to penicillin since that antibiotic came into widespread use.[10–25] Studies in the United States, England and the Scandinavian countries have demonstrated that while more than 80 per cent of these bacteria were sensitive to penicillin prior to 1946, more than 50 per cent of strains now isolated in hospitals are resistant to this antibiotic. It is of interest that highly resistant strains of this microorganism, isolated from patients, are penicillinase producers.[22,23,25] A higher incidence of resistant strains is found in hospital populations than is noted in the community at large.[11–20] These facts would seem to agree with the origin of resistance as the result of a selective action of antibiotics on bacterial populations which vary in their metabolic requirements and genetic constitution.[22,24] Cross infections appear to account for the higher percentage of resistant strains isolated in hospitals.[18–20] A similar loss of sensitivity of the staphylococci to chlortetracycline (aureomycin)[9] and oxytetracycline[17,18] has been noted. However, susceptible organisms are still most sensitive to penicillin if compared on a weight basis with other antimicrobial agents. When resistance occurs, erythromycin (ilotycin® or erythrocin)[26–28] is generally most effective. Carbomycin (magnamycin®)[31] has similar activity but appears less effective weight for weight.[48,56] Resistance to these latter two agents can be produced *in vitro* and has been noted clinically.[21,26–29] Organisms resistant to penicillin may respond to chlortetracycline (aureomycin), oxytetracycline (terramycin), tetracycline (achromycin, tetracyn, etc.), streptomycin (in combinations) or the sulfonamides. It is well to recall that since chloramphenicol (chloromycetin) is used less because of its implication in bone marrow suppression or aplasia, many organisms remain sensitive to it. Cross resistance to certain gram-negative bacteria has been noted

for chloramphenicol (chloromycetin) and the tetracycline group of compounds. However, this occurs less commonly with gram-positive organisms.[95]

Although neomycin and bacitracin are extremely active against the staphylococci, their role in therapy has been largely restricted, by the less toxic antibiotics, to the treatment of bacterial endocarditis or meningitis where their potent bactericidal activity is of advantage. Bacitracin has a nephrotoxicity similar to polymyxin.[32,33] Erythromycin is active against the great majority of staphylococci and is bactericidal for some strains.[30]

Enterococcus: These gram-positive cocci are widely distributed in nature without relation to disease and occur in dairy products as well as the gastrointestinal tract where they are usually present as harmless inhabitants. However, their extreme hardiness is exemplified by their ability to grow on 40 per cent bile agar, in 0.1 per cent methylene blue in milk, and at salt concentrations and temperatures lethal to most other streptococci. They resist pasteurization and produce the group D specific carbohydrate.

Enterococci are not infrequently a problem in genitourinary, respiratory and gastrointestinal infections as well as causing refractory sepsis and bacterial endocarditis. Their resistance to penicillin and sulfonamides is well known and they have demonstrated decreasing sensitivity to aureomycin.[9] Because of their bactericidal activity, streptomycin, penicillin and bacitracin have been particularly helpful in the successful treatment of endocarditis due to enterococci.[51,66,77,96,97,103] When bactericidal *in vitro* activity by erythromycin is demonstrated for a strain, this antibiotic would deserve consideration. While chlortetracycline, oxytetracycline, tetracycline, chloramphenicol and the sulfonamides may be helpful in genitourinary tract infections caused by enterococci, erythromycin, and magnamycin are usually more effective.

Endocarditis: Bacterial endocarditis poses a special problem because the natural history of the disease almost invariably ends in the death of the host. Under such circumstances it is logical to assume that host resistance factors are poor. The relative avascularity of the vegetations, the large tightly packed colonies of bacteria buried in fibrin, the relatively poor phagocytosis, and the lack of adequate antibody response would bear this out. In such a situation the bacteriostatic action of sulfonamides, the tetracycline antibiotics and chloramphenicol has been largely ineffective, and best results have been obtained with bactericidal agents[51,66,77] such as penicillin, streptomycin, bacitracin, polymyxin or neomycin. When bactericidal activity is demonstrated *in vitro*, erythromycin may be helpful. In endocarditis caused by some strains of brucella[54] and pasteurella we have successfully eradicated the infection with the use of aureomycin. However, in these instances this antibiotic was found to be bactericidal *in vitro* for these organisms. Similar destructive activity by primarily bacteriostatic drugs may at times occur against the meningococcus, beta hemolytic streptococcus and other highly sensitive organisms. When markedly bactericidal activity is necessary, as in endocarditis, the inhibition of bacterial metabolism by bacteriostatic chemotherapeutic agents might be expected to interfere with the successful eradication of the infection by bactericidal antibiotics.[59–65,67,68,71,75,76,78] In such a situation, however, the combination of two or more bactericidal compounds may increase the destruction of organisms so that a synergistic effect is obtained.[51,63,65–67,69–75,77,78] The bactericidal activity of any antibiotic is relative and only indicates that when tested *in vitro*, in concentrations obtainable in the body, the agent will markedly decrease the number of viable organisms present.

From the foregoing remarks it may be concluded that in adverse circumstances, if host factors have been evaluated within the scope of present medical knowledge, one may justifiably and successfully employ less common and even potentially toxic agents. However, it is essential that standardized laboratory technics be available which are capable of determining *in vitro* the therapeutic potential of each chemotherapeutic substance. Such a comparison is made possible by use of the tube dilution technic. Sensitivity tests like the agar plate diffusion method are useful only for gross qualitative estimates of activity. They do not allow quantitative estimations, do not permit accurate comparison of the activities of the different antibiotics, or allow ready estimation of bactericidal action.

DRUGS USEFUL IN REFRACTORY BACTERIAL INFECTIONS

Polymyxin (aerosporin): This group of polypeptide antibiotics is produced by the soil bacillus, B. polymyxa or B. aerosporus Greer.[35–37]

Five similar chemical compounds have been recovered from crude polymyxin and labeled A, B, C, D and E. These differ in their amino acid content but all contain L-α,γ, diamino butyric acid and a C_9 fatty acid.[38,80,98,99] In addition, polymyxin A, B and D contain dextrorotatory isomers of amino acids. Since such compounds are not ordinarily found in the animal body, it is thought that this might explain the toxicity noted with polypeptide antibiotics. D-serine is known to produce damage to the renal tubules similar to that caused by polymyxin.[39,100–102] The toxicity of this amino acid is reported to be prevented by administrations of D,L-methionine.[52,104] This latter compound, in our experience,[33] did not prevent damage from polymyxin. Although polymyxin D is the only compound of this group which contains D-serine, the others also produce renal tubular damage. The polypeptide, bacitracin, has a similar toxicity.[53]

Polymyxin is strongly bactericidal and its activity is largely confined to gram-negative bacilli.[8] It is effective against these organisms, with the striking exception of B. proteus, in extremely low concentration. Marked therapeutic activity with only minor toxic manifestations can be obtained with total daily intramuscular doses of 2 to 5 mg. per kg. if the patient is carefully observed.[6,7,86] Such therapy is frequently accompanied by albuminuria, casts and a fall in the specific gravity of the urine. These findings are usually reversible and do not necessitate cessation of treatment. However, a rapid rise in blood urea nitrogen (or NPN) or a marked drop in urinary output to a total daily volume below 500 cc. in adults, is an indication for immediate suspension of therapy. Anuria can be avoided if early signs of oliguria are observed. These nephrotoxic effects are similar for polymyxin, bacitracin and neomycin. Paresthesias (usually perioral), drowsiness, weakness, diplopia, skin reactions, eosinophilia, pain at injection sites, nystagmus, ataxia and febrile reactions may occasionally occur as annoying but temporary minor side reactions that do not require suspension of treatment.[86] Renal tubular damage, fever, hypersensitivity and neurologic effects are reversible when therapy is stopped. However, the greatest care must be exercised in the administration of polymyxin to patients with renal insufficiency since they are most likely to develop anuria and other types of toxicity. Polymyxin,[7,33,80,88,89,110] neomycin[5] and bacitracin[53,105–109] are capable of producing severe lower nephron nephrosis if not discontinued in time. The treatment of this complication is the same regardless of the cause. Neomycin in effective doses is the least likely to cause severe renal damage.

Commercially, polymyxin B is available in 50 mg. sterile vials containing 500,000 units of crystalline powder. The toxicity of polymyxin depends upon the rapidity of administration and the height of the blood levels.[33] Therefore it is important that individual doses do not exceed 1 mg. per kg. This can be accomplished if the antibiotic is given in four to six daily intramuscular injections. These are best diluted in 1 or 2 per cent procaine to reduce local pain. Polymyxin, like streptomycin and bacitracin, diffuses poorly from the blood to the spinal fluid.[33] In meningitis caused by refractory organisms sensitive to polymyxin[43,83,90–92,111–114] this agent should be injected intrathecally, slowly and well diluted in spinal fluid. The total single dose should be kept to 1 to 5 mg. for adults and reduced accordingly for smaller individuals. Doses of 10 mg. injected intrathecally in dogs cause neurologic changes but 1 mg. is without such toxic effect.[33,43] As in the case of streptomycin, neomycin and bacitracin, polymyxin is poorly absorbed from the gastrointestinal tract and may be employed orally for local action on intestinal organisms.[41,42,89,115]

Neomycin: This stable, crystalline, basic antibiotic is produced by the Streptomyces fradiae.[1–5] At least two chemically and biologically different types of neomycin have been identified. They are designated neomycin A and B[45] and are basic compounds most active in alkaline medium, and are thermostable and soluble in water. In low concentration neomycin is markedly bactericidal for gram-negative organisms, and at slightly higher levels it is active against many gram-positive bacteria. Its activity closely parallels that of streptomycin and it is effective against the tubercle bacillus. Fortunately, resistant organisms do not appear as readily as with streptomycin.[1,44] The toxicity of neomycin resembles that of dihydrostreptomycin in producing damage to the auditory portion of the eighth nerve before disturbing vestibular function.[5] The eighth nerve damage may occur several weeks after therapy is discontinued and is not reversible. This complication is more frequent if intrinsic renal disease is present. In addition, neomycin is capable of producing

nephrotoxic effects similar to but less marked than with bacitracin and polymyxin.[5]

Neomycin can be injected intramuscularly if the precautions noted for polymyxin are taken. However, renal and also auditory reactions must be observed. A detrimental change in audiometrics, the onset of oliguria, or a rapid rise in blood urea nitrogen (or NPN) are indications for suspending treatment. As noted for polymyxin and bacitracin, damage is more likely to occur if renal insufficiency is already present, and great care must be exercised since higher blood levels may be present because of faulty excretion. However, when a total daily dose of ten mg. per kg., or approximately 2,000 units per kg., is divided into four equal injections and administered intramuscularly at six-hour intervals no difficulty has been encountered. This is true even in the presence of genitourinary infections. Neomycin and bacitracin have been used simultaneously in the same patient without ill effect. However, the daily urinary output should not be allowed to fall below 500 cc. Neomycin, bacitracin and polymyxin are best dissolved, for intramuscular administration, in 1 or 2 per cent procaine since these antibiotics are irritating. Spinal fluid levels of neomycin are approximately 25 to 50 per cent of the blood concentration following intramuscular injections.[5] As in the case of polymyxin and bacitracin, neomycin is poorly absorbed from the gastrointestinal tract and may be used for its marked local effect on intestinal organisms. In such a capacity it is used to lower the bacterial count in the intestine prior to surgery.[42] However, since some absorption[5,42] does occur, care in this use should be exercised, particularly in the presence of enteritis and colitis in which increased absorption might occur.

Bacitracin: This basic polypeptide antibiotic is produced by a strain of the Bacillus subtilis.[47] It is markedly bactericidal for gram-positive organisms and may be used in combination with penicillin or streptomycin or both in the therapy of refractory bacterial endocarditis.[96,97,103] Bacitracin,[55] like polymyxin, does not readily enter the spinal fluid from the blood and intrathecal injections of 10,000 units may be administered when necessary in the treatment of meningitis.[46] It has a potential nephrotoxicity similar to that of polymyxin and the same precautions must be observed in its use.[32,53,106–109] However, serious toxic manifestations are uncommon when bacitracin, dissolved in procaine, is injected intramuscularly in a daily dose of 1,000 units per kg. This total daily dose is best given at six-hour intervals. Measurement of the twenty-four-hour urinary output and frequent determinations of the blood urea nitrogen will serve as indicators of toxicity. As noted with polymyxin and neomycin, therapy must be discontinued when the excretion of urine is less than 500 cc. per day or the blood urea nitrogen rises rapidly. When these three antibiotics are administered parenterally, some albuminuria, cylindruria and loss of concentrating power of the kidney are common. All toxic manifestations of renal origin are reversible if the agents are stopped at the first sign of oliguria. However, in the presence of intrinsic renal damage great caution must be observed.

Erythromycin (ilotycin and erythrocin) and *carbomycin* (magnamycin): These are two of the newer antibiotics whose chemotherapeutic spectrum resembles that of penicillin.[26–31] However, in addition to their activity against gram-positive bacteria they also have an effect against rickettsiae and certain of the large viruses. They are basic crystalline compounds which are stable. Erythromycin is a product of the Streptomyces erythreus and carbomycin is formed by the Streptomyces halstedii. They have a similar pattern of activity and show cross resistance[48] so that bacteria made resistant to one become resistant to the other. Whether they are chemical analogs, such as the tetracycline drugs[49,57] which demonstrate the same phenomenon, is not determined; however, cross resistance for some organisms occurs with chemically unrelated antibiotics, such as the tetracycline drugs and chloramphenicol.[95] Usually more carbomycin than erythromycin is needed to inhibit a sensitive organism and reports of clinical trials have not been as favorable for carbomycin.[48,56]

Both erythromycin and carbomycin are of low toxicity. Diarrhea, nausea, vomiting and skin reactions similar to those observed with chlortetracycline, oxytetracycline and chloramphenicol have been noted as undesirable but not dangerous side effects. Erythromycin has demonstrated bactericidal as well as bacteriostatic[30] action for some strains of organisms. This "cidal" effect may occasionally be helpful in the therapy of bacterial endocarditis. However, the most striking asset of these antibiotics is observed with staphylococci and streptococci which, although resistant to penicillin, streptomycin, oxytetracycline, tetra-

cycline, chlortetracycline, chloramphenicol and the sulfonamides, are usually sensitive to erythromycin and carbomycin.[26–31,116,117] The development of resistance to erythromycin might be minimized by using it in combination with other antibiotics. Both antibiotics are usually well tolerated in total daily oral doses of 20 to 40 mg. per kg. administered at four- or six-hour intervals.

Furadantin (N-(5-nitro-2 furfurylidene)-1-aminohydantoin): This chemotherapeutic agent is a yellow, stable, crystalline compound which has such desirable qualities as low toxicity, little tendency for the emergence of resistant organisms, and a broad antibacterial spectrum. It is active against both gram-positive and negative bacteria but it is not effective for viruses, fungi or rickettsiae. Some effect on protozoa has been described.[50]

A considerable number of patients develop nausea and vomiting while taking this drug, and this appears to be the major toxicity noted to date. The blood concentrations achieved with the usual daily dose of 5 to 10 mg. per kg. are extremely low and limit this agent to the therapy of infections of the genitourinary tract since furadantin is excreted in high concentration in the urine. It has proved helpful in the therapy of some refractory urinary tract infections,[50,118–126] particularly those due to B. proteus. It does not usually show cross resistance with the sulfonamides or antibiotics.

SUMMARY

Since the introduction and widespread use of antibiotics, organisms such as B. proteus, Ps. aeruginosa, coli-aerogenes, Staph. aureus and the enterococcus have emerged as common etiologic agents of refractory infections. With proper precautions and careful observation, more toxic antibiotics such as polymyxin, neomycin and bacitracin may be successfully employed in the treatment of these infections. In addition, erythromycin, carbomycin and furadantin are more recent chemotherapeutic agents which are of some assistance in combating organisms resistant to the older antibiotics. Of course, it must be stressed that the successful treatment of a refractory bacterial infection depends on the early and proper evaluation of the diagnosis and the host factors involved, as well as consideration of the chemotherapeutic and bacterial agents. The answer to eradication of these infections will most frequently be found in sound clinical judgment, application of long-established principles of general medical and surgical therapy, and in carefully standardized and controlled laboratory sensitivity determinations.

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Complications of Antibiotic Therapy*

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Few drugs have proved to be of such value in the treatment of infectious conditions as the antibiotics, and there are few persons who have not benefited from them. Unfortunately, however, there exists some misconception among the laity and the medical profession as to their innocuousness, the potential dangers connected with their use, and their proper use under various conditions. Antibiotics are no panacea for all kinds of infectious conditions, they have to be selected properly according to the type of infection and their administration has to be controlled carefully in order to avoid complications. The potential dangers resulting from the injudicious use of antibiotics are becoming more and more recognized, as indicated by numerous publications such as those of Gilman (1950), Smith (1952), Hall (1952), Herrell (1953), Bickel and associates (1953), Blanton and Blanton (1953), Robinson (1954) and Reimann (1953). The latter cautioned especially against the indiscriminate use of antibiotics for such trivial conditions as the common colds, 95 per cent of which are due to virus infections which do not respond to antibiotics. An editorial to the paper of Bell and coworkers (1954) cautions specifically against the use of penicillin for the prevention of postsurgical infections which are of low incidence, especially if all aseptic precautions are observed. Antibiotics should not be used for trivial infections.

Probably the greatest danger and the most serious complication in the use of antibiotics is the possibility of *sensitizing* the patient to these drugs and the occurrence of anaphylactoid reactions. The incidence of such reactions has been estimated by different investigators at different levels. For penicillin, Berkowitz and associates (1953) estimated it as 2 per cent in allergic children, Berry and Ferber (1954) as 0.36 per cent after oral administration, and Krasno and collaborators (1948) as 3 to 6 per cent after inhalation. Other observers such as Risman and Boger (1950), Flinn and associates (1945), Kolodny and Denhoff (1946), Feinberg (1952), Duemling (1946) and Barksdale (1946) gave the incidence of reactions as 6 to 12, 3, 16, 2 to 5, 10 and 8 to 10 per cent.

Of great significance is the report of Peck and his coworkers (1948) who noted only 5.4 per cent allergic reactions in patients without previous medication with penicillin as compared with 25.4 per cent after previous medication with this drug. Berkowitz and associates (1953) reported 0.7 per cent allergic reactions with aureomycin and oxytetracycline in allergic children and Keefer and associates (1946) reported 0.4 per cent incidence with streptomycin. It appears further that the mere handling of antibiotics by physicians and nursing personnel may result in sensitization. In 1953 the British Ministry of Health reported an incidence of 3.5 per cent among nurses and Amsler (1951) reported that in fifty-seven of seventy nurses handling streptomycin some allergic reaction developed. It is therefore apparent that an appreciable number of patients are or will become sensitive to various antibiotics.

The most serious reaction of this type is *anaphylactoid shock*. As illustrated in Table 1, a total of eighty-three severe and forty-six fatal anaphylactoid reactions have been reported after medication with penicillin preparations, three severe and one fatal reaction after the use of streptomycin, and two severe and one fatal after dihydrostreptomycin, and one severe reaction after chlortetracycline. It appears significant that reports on such reactions to penicillin have increased in recent years, whereas they are rather limited after medication with streptomycin, the use of which is considerably less widespread. It is further evident that the incidence of anaphylactoid reactions from penethamate (neo-penil®) and procaine-penicillin is relatively high. According to Welch and associates (1953), penicillin is more apt to

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cause anaphylactoid reactions when injected than after oral administration. As pointed out by the same investigators and as supported by other reports, patients with bronchial asthma, hayfever and other allergies, or those previously sensitized to penicillin, are especially prone to exhibit anaphylactoid reactions.

TABLE I

OCCURRENCE OF ANAPHYLACTOID SHOCK AFTER MEDICATION WITH ANTIBIOTICS

Author	No. of Severe Reactions	No. of Fatal Reactions	Antibiotic
Wilensky (1948)	..	1	Penicillin
O'Donovan and Klorfajn (1946)	1	..	Penicillin
Burleson (1946)	1	..	Penicillin G
Waldbott (1949)	..	1	Penicillin
Corajod et al. (1951)	..	1	Penicillin
Irwin et al. (1951)	1	..	Procaine-penicillin G
Everett (1951)	1	..	Penicillin G
Yuval (1952)	1	..	Procaine-penicillin
Wylie-Smith (1952)	1	..	Penicillin
Strout (1952)	1	..	β-Ephenamine-penicillin G
Thomson (1952)	..	1	Penicillin
Higgins and Rothchild (1952)	..	1	Penicillin G
Harpman (1952)	1	1	Procaine-penicillin
Welch and associates (1953)	37	18	Procaine-penicillin
Welch and associates (1953)	21	5	Phenetamate
Welch and associates (1953)	..	1	Dibenzylethyleneamine-dipenicillin G
Weiss (1953)	1	..	Crystalline penicillin G
Sohval (1953)	1	..	Penicillin G
Siegal et al. (1953)	2	1	Procaine-penicillin
Nikishin (1953)	1	..	Penicillin
Mignault and Mitchell (1953)	1	..	Procaine-penicillin
Mayer et al. (1953)	5	1	Procaine-penicillin, Penicillin
Icasiano (1953)	1	..	Penicillin G
Curphey (1953)	..	2	Procaine-penicillin, Penicillin
Chapman and Metheney (1953)	..	1	Procaine-penicillin
Blanton and Blanton (1953)	..	1	Penicillin G
Pick and Patterson (1953)	..	1	Penethamate
Bell (1953)	1	..	Crystalline penicillin
Ruskin (1954)	1	..	Penicillin (skin contact)
Nemser (1954)	1	..	Penicillin
Rosenthal (1954)	..	8	Penicillin
Fisher (1954)	..	1	Penicillin
Welch et al. (1953)	2	1	Streptomycin (intrathecal)
Welch et al. (1953)	1	..	Streptomycin (injection)
Mark (1950)	2	..	Dihydrostreptomycin
Levi-Valensi and Molina (1951)	..	1	Dihydrostreptomycin
Gittel (1951)	1	..	Chlortetracycline

Skin eruptions because of allergy are not uncommon after medication with antibiotics. Whereas, according to Keefer and associates (1943), the incidence of side reactions after penicillin was low in the first years of its therapeutic use, there has been a considerable increase in such reactions in the years that followed, and as pointed out by Jenkins (1953), a further increase with the present widespread and injudicious use of the drug. It appears that topical applications are especially prone to cause sensitization. Such cases were reported for penicillin by Kolodny and Denhoff (1946), Schirmer (1952) and Feinberg (1952). As pointed out in a report of the Council of Pharmacy and Chemistry (1947)

contact dermatitis from exposure to streptomycin occurs frequently. According to a survey made by the British Ministry of Health (1953), 256 cases of sensitization to antibiotics were discovered among the nursing staff of seventy local health agencies and seventy-six chest hospitals and sanatoria, corresponding to an incidence of 3.5 per cent. Of these, 188 were sensitized to streptomycin, eighty to penicillin and two to chloramphenicol and p-aminosalicylic acid. In the majority of the cases hands, arms, eyes and the neck were affected. Schirmer (1952) reported such a case following exposure to penicillin, and Rosen (1948), Amsler (1951), L'Epée and Texier (1951) and Gauthier (1952) after contact with streptomycin. An editorial in the *British Medical Journal* (1953) gives detailed directions for the safe handling of antibiotics as formulated by a committee of experts. Similarly, allergic dermatitis may also be encountered in the manufacture of penicillin and streptomycin, as reported by Mejer (1949) and Roberts (1953), respectively. According to Feinberg (1952) allergic reactions following skin contact with penicillin or its topical application are so numerous that the latter method of penicillin administration has practically been abandoned. A similar opinion was expressed by Jenkins (1953) and such cases were reported by Markson (1945), Kolodny and Denhoff (1946), Krasno, Carp and Rhoads, (1948) and Katzenstein (1949). Cutaneous eruptions may run the whole gamut from erythema to papular, vesicular, bullous, morbilliform and eczematous eruptions, as reported by various authors with different antibiotics, most commonly with penicillin, as indicated by Graves and associates (1944), Cormia and coworkers (1945), Samitz and Horvath (1949) and others. Occasionally, exfoliative dermatitis may occur, as reported by Nolan and Pedigo (1946), Barksdale, Frost and Nolan (1948), Rabinowitch and Snitkoff (1948), Berne (1950), and Langdon (1950) with penicillin; by McKechnie (1953) with streptomycin; and by Fein and Criep (1950) with dihydrostreptomycin.

Urticarial eruptions are most commonly observed with penicillin, as reported by Keefer and coworkers (1943), Cormia and associates (1945), Barker (1945), Davis (1947), Dressler and Dwork (1947), Barksdale and collaborators (1948), Felder and Felder (1950) and Nemser (1954). They have been observed less frequently after chlortetracycline, as reported by Daugherty (1952), and after streptomycin, as observed by Mark (1950).

Angioneurotic edema subsequent to medication with penicillin has been reported repeatedly as by Scheinberg (1946), Felder and Felder (1950) and Riley (1952).

Fixed drug eruptions have been reported occasionally, as by Baker (1945) after penicillin; by Welsh and Goldberg (1951) after chlortetracycline, and by Welsh (1952) after oxytetracycline.

Most of the allergic skin reactions have been observed after repeated medication with antibiotics or in patients with a history of allergy and, as pointed out by Feinberg (1952), patients with fungous infections of the skin may respond frequently to penicillin with vesicular eruptions and exfoliative dermatitis. This seems to be supported by the report of Graves, Carpenter and Unangst (1944) on vesicular eruptions following treatment with penicillin of two patients who gave a positive reaction to trichophytin and by the observation of Basex and Bouisson (1947) of erythemovesicular eruptions at the site of existing fungous infections. Similarly Barksdale and associates (1948) noted an exacerbation of epidermophytosis and of epidermophytids after medication with penicillin, and Peck and collaborators (1948) found that spontaneous reactions to penicillin were three times as frequent in patients with a positive trychophytin test than in those with a negative test. According to Kolodny and Denhoff (1946), 25 per cent of patients suffering from dermatologic conditions showed an immediate reaction to medication with penicillin as compared with an incidence of 6 per cent among patients with normal skin. Sanchez-Cuenca (1950) reported on allergic reactions in patients who harbored Penicillium fungi in their feces, in one instance in the sputum, one of whom could be desensitized with an extract of Penicillium spores. As to the incidence of skin reactions to different antibiotics, these seem to be rather common with penicillin and streptomycin; according to Crissey and Caccamise (1953), they are uncommon with chloramphenicol and chlortetracycline and are rarely observed with oxytetracycline.

Other allergic reactions characterized by *rhinitis*, *conjunctivitis* and *bronchial asthma* have been reported much more frequently with penicillin than with other antibiotics, as pointed out by Jenkins (1953). Such cases were reported by Bissel (1946), Leibowitz and Schwartz

(1950) and Roberts (1953). Gauthier (1952) reported one such case after medication with streptomycin.

Drug fever has been reported repeatedly after medication with antibiotics. According to Herrell (1953) it is rather common after medication with penicillin, possibly because of contamination in some manner with pyrogens. Buggs and associates (1946) observed drug fever in three of forty-five patients after medication with streptomycin, and Yow and Moyer (1953) in seven of thirty-nine patients treated with polymyxin B.

Serum sickness-type reactions, characterized by urticaria, angioneurotic edema, arthralgia, swelling of the joints and fever starting after an inoculation period of several days and lasting usually one to two weeks, have been reported frequently after medication with penicillin, as reported by Cormia and associates (1945), Haswell and Wilkinson (1946), Mendell and Prose (1946), Davis (1947), Wells and Meyers (1948), Grimmer (1950), Feinberg (1952), Hall (1952), Jenkins (1953) and Mayers and coworkers (1953) who estimated the incidence as 6 per cent among patients treated with this drug. It is frequently observed after repeated administration or in patients with a history of allergy. Johnston and Cazort (1953) reported one case after oral administration of oxytetracycline.

Injury of the blood-forming organs may also be the result of an allergic reaction of the bone marrow and is not uncommon with certain antibiotics whereas it is less frequent with others. Stötter (1952) observed one case of leukopenia among 120 patients treated with oxytetracycline. This patient improved after discontinuance of the drug. As stated in a report of the Council on Pharmacy and Chemistry (1947), the incidence of agranulocytosis after medication with streptomycin is low, at that time 0.98 per cent; single cases were reported by McDermott (1947), Benhamou and associates (1948), Feld (1949) and Pallister (1949). This complication appears also to be exceptional with administration of penicillin, a single case being reported by Spain and Clark (1946). Volini and associates (1949), Gill (1950) and Robinson and collaborators (1952) each reported two cases of agranulocytopnia after medication with chloramphenicol and one instance of aplasia of the granulocytic bone marrow which was noted two days after discontinuance of the drug.

Aplastic anemia has been reported repeatedly after medication with chloramphenicol. Lewis and associates (1952) stated that of 539 cases of blood disorders, fifty-five occurred after the use of this antibiotic and forty-four of these suffered from aplastic anemia which in twenty-three patients ended fatally. Single cases of aplastic anemia following chloramphenicol were reported by Rich and coworkers (1950), Freedman (1952), Willis and collaborators (1952), Winternitz (1952), Wolman (1952), Sturgeon (1952), Wilson and associates (1952), Loyd (1952), Hollis and Hanish (1953), Pickard and Rosenblatt (1954) and Johnston (1954). Two cases each were reported by Hawkins and Lederer (1952), Clandon and Holbrook (1952), and two and possibly three were published by Smiley and collaborators (1952). Janbon and Bertrand (1951) reported six cases, Rheingold and Spurling (1952) five, and ten cases with seven fatalities were published by Hargraves and associates (1952), who referred to two additional cases. As pointed out by these authors the aplastic anemia may result from sensitization of the patient to chloramphenicol inasmuch as the first course is frequently well tolerated and only the second course precipitates exhaustion of the bone marrow. Aplastic anemia may less frequently result from an existing hypersensitivity and in such cases it is precipitated by a single course of medication. From these reports it appears that single short courses are generally well tolerated but that chloramphenicol should not be used for prolonged administration or for repeated courses. In addition it seems that children are more sensitive than adults. Aplastic anemia has also been observed, in very few instances, after prolonged medication with streptomycin, as reported by Deyke and Wallace (1948), Womack and Reiner (1951) and Sacks and collaborators (1951).

Janbon and Bertrand (1951) reported six cases of hemolytic anemia after prolonged medication with chloramphenicol.

Keefer and associates (1946) reported one case of hemorrhagic purpura following medication with streptomycin and McDermott (1947) and Rudensky and Fisher (1951) each published one case of thrombocytopenia after medication with this drug. Following treatment with chloramphenicol Sabbatini (1951) noted in eight of ten patients retardation of the coagulation time and a reduction of the prothrombin level in the blood to 30 to 60 per cent of the normal. Dameshek and

Campbell (1952) reported one case of thrombocytopenia and hypoplastic anemia.

Eosinophilia as a sequel to the use of antibiotics has been observed after prolonged administration of penicillin, as reported by Berk and Sostek (1948). As pointed out in a report of the Council on Pharmacy and Chemistry (1947), some degree of eosinophilia is frequently seen after medication with streptomycin and, according to Mark (1950), it may be present in 95 per cent of the patients. McDermott (1947) stated that the percentage of eosinophils may reach 10, 15, 25 and even 40 per cent. von Gerzantis (1952) reported values as high as 50 per cent.

Herxheimer reactions are said to be relatively frequent in syphilitics treated with penicillin. Barksdale and associates (1948) reported their incidence as 30 per cent, Duemling (1946) as 50 per cent and Morginson (1946) as high as 86 and 91.6 per cent. In some patients these may represent serious complications. Diefenbach (1949) reported on a patient who as a result of this reaction died with symptoms of complete bronchial occlusion and dilatation of an existing syphilitic aneurism. Another fatality was reported by Rotter and Wagner (1952).

A rare complication in syphilitic patients treated with penicillin is *epididymitis*, as reported by Rosenberg and Arling (1944) and by Cormia and associates (1945).

Nelson and Braslow (1953) reported one fatal *Shwartzman reaction*, characterized by extensive hemorrhages and gangrenic ulcerations, in an infant after a second course of penicillin medication.

As to the *possibility of cross-sensitization* to various antibiotics, the studies of Risman and Boger (1950) and of Siegal (1951) indicate that this may hold true to some extent for various penicillin preparations although, as shown by Marsh and Tillotson (1951), this does not seem to be a strict rule.

As to the mechanism of allergic reactions, the following possibilities were suggested by Goltman (1952), also in the reports cited: (1) Transfer of sensitization in utero. (2) In exceptional cases, an antigen-antibody reaction. (3) Perhaps more frequently, hapten formation. (4) A common antigen effect due to the presence of human mycoses, such as tinea pedis, capitis or crurae etc., which may predispose to allergic reactions to antibiotics by common protein effect and which may account for vesicular eruptions resembling fungous diseases and which may be seen in various locations after antibiotics. (5) Hypersensitization because of inhalation of spores from molds in household articles, closely related to those molds from which antibiotics are made. (6) It may result from ingestion of certain cheeses of the Rochefort, Camembert and Gorgonzola type, or of moldy bread or of other moldy food. (7) And finally it may be due to the presence in the body of molds related to the antibiotic, specifically in pulmonary abscesses or in the intestinal tract.

It is therefore apparent that apart from the sensitization produced by repeated medication with antibiotics and an existing allergic predisposition, a number of other factors may be involved in allergic reactions to antibiotics. This indicates the necessity of careful interrogation of the patient with regard to such possibilities before antibiotics are administered, as was pointed out by Kern and Wimberley (1953), Feinberg and associates (1953) and Rosenthal (1954). Curphey (1953) emphasized that in patients with an allergic diathesis antibiotics should be among the last rather than the first remedies to be chosen. To prevent such accidents, testing for hypersensitivity to antibiotics by skin or scratch testing is indicated, as recommended by Mayer and associates (1953). It should, however, be pointed out that patch testing may give negative results in spite of an existing allergy. Scratch and intradermal tests are more reliable but, as shown by Rosen (1948) and by Blanton and Blanton (1953), serious reactions may be produced in hypersensitive patients if these tests are not performed with adequate precautions. This is illustrated by the report of Schirmer (1952) who saw a violent reaction after an intradermal dose of 400 U. of penicillin and by Rosen (1948) who observed a severe anaphylactoid reaction after the intracutaneous administration of 5,000 U. of streptomycin; for this reason he suggested for this antibiotic an initial dose of 100 U. which, if negative, should be increased step by step before the full dose is given. Waldbott (1949) proposed starting the intradermal test with 100 to 300 U. of penicillin and, if no reaction occurs after fifteen minutes, repeating the test with 5,000 U. It may even be desirable to increase the test dose to 10,000 and 20,000 U. and to give the full dose if no reaction occurs within four hours, as suggested by Pillsbury and associates (1947), who further advised keeping benadryl® and epinephrine on hand in case untoward reactions

should occur. As shown by O'Donovan and Klorfajn (1946), and Roberts (1953), hypersensitive patients may be desensitized by the injection of gradually increasing doses.

In addition to allergic reactions, certain antibiotics may cause other side reactions which in part are intrinsic to the compounds or may be phenomena secondary to allergic reactions or other complications caused by these agents. In the experience of Kutscher and associates (1952), the incidence of side reactions after medication with oxytetracycline given in the form of troches was 53.2 per cent; in 14.7 per cent they were sufficiently severe to warrant discontinuance of the medication. Similarly, Kutscher and coworkers (1953) noted in 51 per cent of patients treated with chlortetracycline untoward reactions which in 14 per cent were sufficiently severe to necessitate discontinuance of the treatment.

With certain antibiotics *gastrointestinal disturbances*, characterized by nausea, vomiting and diarrhea, are not uncommon. While these usually are not very serious, they may be sufficiently severe at times to warrant discontinuation of the drugs and in critically sick patients they may represent a serious complication and considerably aggravate the general condition. Nausea and vomiting are seen only occasionally after medication with penicillin, streptomycin and tetracycline, as reported by Spring (1951), by the Council on Pharmacy and Chemistry (1947) and by Waddington and collaborators (1954). Nausea and vomiting were reported after medication with oxytetracycline by Herrell and coworkers (1950), Wilcox and Findlay (1952), Stötter (1952), Miller and Walker (1953), and Sayer and collaborators (1951). According to Bottman and associates (1953) gastrointestinal upsets are less frequent after administration of the amphoteric compound than after the hydrochloride. They are frequently encountered after administration of chloramphenicol, as reported by Harris (1950), Mukerjee and Mitra (1950) and Cerny and Ochsé (1951), but they are evidently quite frequent after oral administration of chlortetracycline as judged by the reports of Randall and associates (1949), Meyer (1950) and Harris (1950).

In many instances nausea and vomiting are associated with *diarrhea*. This seems to be exceptional with streptomycin and it has been reported in single cases with penicillin sulfonamide by Merliss and Hoffman (1951) and with penicillin by Spring (1951). It has been observed frequently after medication with oxytetracycline by Timpanelli and associates (1950), Merliss and Hoffman (1951), Sayer and coworkers (1951), Wilcox and Findlay (1952) and Boltman and collaborators (1953). It has also been reported after medication with chloramphenicol, as by Smadel (1949), Merliss and Hoffman (1951), Catanzaro and associates (1952), von Gerzantis (1952), Simard and associates (1953) and Harris (1950). According to the latter author, diarrhea is, however, much more frequently seen with chlortetracycline and such cases were reported by Meyer (1950), Welsh and Goldberg (1951), Gittel (1951), Vayssière and Simard (1951) and Merliss and Hoffman (1951). Simard and collaborators (1953) reported four and Gardner (1953) two cases of enterocolitis after medication with chlortetracycline. Waddington and collaborators (1954) reported diarrhea in a small number of patients after medication with tetracycline. The mechanism by which this diarrhea is produced has not been definitely established and it may vary with different patients and different antibiotics. In the opinion of Merliss and Hoffman (1951) the clinical picture is very similar to diarrhea observed in sprue and presumably is due to a state of vitamin deficiency. In other instances it may be due to the preponderance of antibiotic-resistant pathogenic strains of microorganisms and fungi, as will be discussed subsequently.

This change of the bacterial flora in the intestinal tract is probably also involved in the appearance of stomatitis and glossitis which are frequently seen after medication with troches containing oxytetracycline, as reported by Kutscher and associates (1953), and after the administration of chlortetracycline, as seen by Müller and Vogt (1951) and Pappenfort and Schnall (1951). It has been reported in single cases after medication with chloramphenicol by von Gerzantis (1952), after penicillin and procaine-penicillin by Krasno and coworkers (1948), after oxytetracycline by Kutscher and coworkers (1952), and after medication with streptomycin by Pallister (1949), Amsler (1951) and Fischer (1951). In cases of blood dyscrasias, stomatitis may be associated with ulceration and in other patients this may be due to changes of the bacterial flora, as will be discussed further.

Pruritus of the anal and perianal regions appears to be frequent after medication with chlortetra-

cycline, as reported by Müller and Vogt (1951), Pappenfort and Schnall (1951), Welsh and Goldberg (1951) and Sidi and Plas-Arditti (1954), and it is quite frequently seen after administration of oxytetracycline, as observed by Bottman and associates (1953); whereas it has been evidently less frequently reported after chloramphenicol, penicillin and streptomycin.

Occasionally, the use of antibiotics, especially in the form of troches, has resulted in the appearance of "black tongue," as reported by Ronchese and Kern (1953) after the use of chlortetracycline ointment around the mouth and by Tomaszewski (1953) after chlortetracycline and also after medication with chloramphenicol and oxytetracycline. Bartels (1953) considered the latter at least a contributing factor in the genesis of this condition.

Other side effects of antibiotics on the digestive organs include *injury of the liver*. This, however, is less common and as a rule not serious. It has been reported most frequently after intravenous medication, especially after excessive doses of chlortetracycline, as indicated by the studies of Rutenberg and Pinkes (1952) and as reported by Lepper and associates (1951), Yessner and Kunkel (1951), and Saint and Joske (1953). One such case was reported by Köhler and Kleinfelder (1950) after administration of penicillin and by Minet and coworkers (1950) after streptomycin, whereas Roost and Schmid (1950) considered dihydrostreptomycin at least a contributing factor in the aggravation of an existing liver injury.

Similarly, *injury to the kidneys* is not common and has been reported only occasionally in single cases, as by Bateman and associates (1952) after penicillin and by Spring (1951) after procaine-penicillin. According to a report of the Council on Pharmacy and Chemistry (1947), it may be seen after medication with streptomycin but is then usually not sufficient to warrant discontinuance of the drug. According to McDermott (1947), it is mostly seen in patients with renal impairment. Under similar conditions transient albuminuria has also been reported after medication with polymyxin B, as by Kaplan and coworkers (1949) and Yow and Moyer (1953), but according to Wallerstein and Schoenbach (1949) this may be avoided by careful dosage. On the other hand, the intramuscular administration of bacitracin is frequently followed by renal irritation, as reported by Zintel and associates (1945 and 1949) and Genkins and coworkers (1954). According to Bateman and associates (1952), large intravenous doses of oxytetracycline may cause azotemia and hypertension, indicating the possibility of a nephrotoxic action.

Injury of the peripheral nerves near the site of the injection after administration of penicillin was reported by Kolb and Gray (1946), Barksdale and coworkers (1948) and Broadbent and collaborators (1949) when the injections were made adjacent to a peripheral nerve. Pulaski and associates (1949) frequently noted neurologic disturbances, such as paresthesias, after intravenous injection of polymyxin B. Long and coworkers (1953) reported that intramuscular injection of the calcium chloride complex of streptomycin caused local pain in some patients which was rarely the case when streptomycin sulfate was used. Philpott and collaborators (1954) reported on a patient who developed nodules of sarcoid granulomatous character at the site of the injection of penicillin several weeks later.

Toxic manifestations referable to the central nervous system have been reported after intrathecal and intraspinal injections of penicillin by Morginson (1946), Chou and Welply (1950) and Bockel (1953). They were characterized by muscular twitchings, clonic spasms, stiffness in the neck, convulsions, unconsciousness, coma and, finally, vascular collapse. Following this type of administration of penicillin a clinical picture similar to meningism was reported by Riccabona (1950), Edwards and Kelsey (1950) and Beikert and Noetzel (1952), and after injection of streptomycin by Bertoye and associates (1950). Psychoses, characterized by restlessness and hallucinations probably of allergic origin, were reported after medication with penicillin by Kline and Highsmith (1948) and Corcoran (1950). According to Zintel and associates (1945), large doses of streptomycin may cause temporary headache and Hunnicutt and coworkers (1948) reported on a patient who reacted to repeated injections of streptomycin with paresthesias, athetotic and choreiform movements, high fever and coma. McKay and associates (1951) noted in fourteen of ninety patients serious reactions characterized by a shock-like state with convulsions and signs of cerebellar injury after intrathecal injections of the same antibiotic. Kaplan and coworkers (1949) observed lethargy, irritability and anorexia after medication of children with polymyxin B. Yow and

Moyer (1953) saw a high incidence of transient paresthesias after medication with this drug. Pulaski and associates (1949) reported that after intravenous injection of polymyxin B mild dizziness and weakness occurred frequently, disappearing twenty-four hours after the last dose.

Visual disturbances following medication with antibiotics are rather exceptional and transient, as reported by Harris (1950) with the use of chloramphenicol. Sannella (1953) after prolonged medication with streptomycin noted visual disturbances characterized by movement of objects in the visual field on rotation of the head. These preceded the vestibular disturbances produced by this drug and became more severe as the latter became more pronounced.

Vestibular and auditory disturbances are observed frequently after medication with streptomycin and dihydrostreptomycin. Hinshaw and Feldman (1945) and Brown and Hinshaw (1946) were apparently the first to report on these reactions. According to a report of the Council on Pharmacy and Chemistry (1947), these are the most common disturbances following the administration of streptomycin, 96 per cent of the patients suffering from vertigo after four weeks of medication with this drug in doses of 1.8 and 2.0 gm. In the experience of Mark (1950), daily doses of 1 gm. given in two fractional doses of 0.5 gm. each, cause vestibular disturbances of variable intensity in 44 per cent of the patients, and Jonghees and Hulk (1951) pointed out that especially in patients with renal impairment even small doses may cause vestibular disturbances, differences in susceptibility being partly due to variations in the level of streptomycin in the blood; this was pointed out also by Carr and associates (1950). In the opinion of Jonghees and Hulk (1951), the impaired vestibular function may be restored in 5 per cent of the patients. As pointed out by McDermott (1947), the auditory branch of the eighth nerve is less seriously affected by streptomycin than the vestibular apparatus. Moffit (1948) showed by audiometric measurements that daily doses of 1 to 2 gm. cause no definite hearing loss such as occurs with doses of 3 gm. With dihydrostreptomycin, however, the opposite holds true, as shown by Bernard and associates (1950), Roland (1951), Grant (1951), Heck and associates (1953) and Minkenhof and von Deinse (1954), and this drug is more likely to cause permanent deafness than streptomycin. As pointed out by Biagi (1951) and Harrison (1954), impairment of hearing may become apparent after the drug has been stopped, it cannot be anticipated, it may progress very rapidly and may become permanent; nor can it be prevented by discontinuing the medication. In the opinion of Winston and associates (1948), the intensity of the injury depends upon the daily dose and the duration of the medication but this appears not always to be the case.

As to *cardiac complications*, these appear to be exceptional and are mostly associated with allergic reactions. Felder and Felder (1950) reported a case of myocarditis after urticaria and angioneurotic edema subsequent to penicillin medication. Grandjean (1951) saw cardiac infarcts in two elderly persons following urticarial eruptions eight and ten days after the beginning of penicillin therapy. Glotzer (1954) reported on electrocardiographic changes during sensitivity reactions caused by penicillin. These were characterized by changes in the T-S interval and T waves, consistent with pericardial involvement, which returned to normal within a few days after discontinuance of the drug. In his opinion such changes are not as rare as would appear from the few cases reported in the literature.

It is, therefore, evident that apart from the danger of allergic reactions, certain antibiotics, especially streptomycin and dihydrostreptomycin, may cause serious toxic effects.

Another aspect of medication with antibiotics is their *effect on the intestinal bacterial flora* of the patient. The suppression of pathogenic organisms by antibiotics and the resulting decrease in danger from infection during and after surgical procedures involving the opening of the intestinal lumen has resulted in the routine prophylactic use of antibiotics in these and other operations. This custom may cause serious complications apart from allergic reactions. Smith (1952) pointed out that prolonged medication with penicillin suppresses or eliminates gram-positive bacteria and directly or indirectly stimulates the multiplication of gram-negative bacilli. On the other hand, prolonged therapy with relatively large doses of streptomycin may suppress the gram-negative bacilli and stimulate the growth of gram-positive cocci, this effect not being as constant as the reverse one induced by penicillin. According to Bottman and associates (1953), coliform bacteria disappear from the intestinal tract within seventy-two

hours after medication with 750 mg. of oxytetracycline every six hours for a total of 3 gm. Dearing and Needham (1953) found a combination of neomycin and oxytetracycline extremely effective in eliminating culturable aerobic and anaerobic organisms from feces. As will be shown subsequently, prolonged suppression of bacterial growth in the intestine may produce certain undesirable effects, and in addition may favor the production of antibiotic-resistant strains and thus decrease the therapeutic efficacy of the antibiotics administered.

As pointed out by Dearing and Fordyce (1953), the presence of antibiotic-resistant pathogenic strains in the intestine in more or less pure culture may produce gastrointestinal and sometimes systemic reactions which occasionally are quite severe unless controlled by other antibiotics. Such cases were reported by Hall (1952), Mooney (1953), Haubold and associates (1953b), Simard and collaborators (1953), Senn (1953), Hay and McKenzie (1954) and Stillwell (1953) who pointed out that 40 per cent of the strains of Micrococcus pyogenes produce rather large quantities of potent enterotoxic substances which may give rise to such clinical symptoms as vomiting, profuse watery diarrhea, fever, hypotension, anuria and uremia. This question of "superinfection" was reviewed by Weinstein, Goldfield and Chang (1954) who studied its incidence in 3,095 patients under medication with different antibiotics. They found that 2.19 per cent developed superinfections and, in cases of pertussis, as many as 20 per cent. After medication with penicillin superinfections were mostly due to gram-negative bacteria; Staphylococcus aureus was frequently the cause after penicillin and streptomycin had been given. In addition it was found that in an appreciable number of cases the superinfecting organism was relatively insusceptible to the available antibiotics and that the superinfection frequently presented a much more serious problem than the original infection and in few instances proved untreatable and fatal. In most instances the superinfection involved the same organ as the primary infection especially when the latter involved the lungs or the middle ear. It occurred most frequently on the fourth or fifth day of antibiotic therapy but some times as late as one week or more. Predisposing factors appear to be an age of three years or less, and the use of broad-spectrum antibiotics. They found that the incidence of superinfection after prophylactic medication in poliomyelitis and measles resulted seven and sixteen times, respectively, more frequently in superinfections than the average of the whole group, and it was quite frequent after medication with chlortetracycline, oxytetracycline and chloramphenicol.

As pointed out by Smith (1952), prolonged medication with penicillin and streptomycin, of chlortetracycline and chloramphenicol, or oxytetracycline may promote the *multiplication of yeast and mold-like fungi* from the normal ecologic flora in the intestinal tract to such a degree that fungous diseases may result in the mouth, intestinal tract, bronchi, lungs and around the genital organs. In exceptional cases such infections may spread through the entire organism. Thus Brown and associates (1953) reported five fatal fungous infections with Candida albicans, Candida tropicalis and other yeast-like organisms which followed the use of several antibiotics. In their opinion medication with broad-spectrum antibiotics should be carefully controlled for evidence of fungous invasion, because they appear to be especially likely to give rise to such complications. Pappenfort and Schnall (1951) reported cases of stomatitis and perlèche evidently caused by C. albicans following oral medication with chlortetracycline, Hall (1952) saw secondary infections in the mouth and intestinal tract, and Bartels (1952) reported on Monilia infections in the mouth after medication with antibiotics. Furred or black tongue occurring subsequent to the administration of antibiotics, presumably because of excessive growth of candida, was reported by Harris (1950), Ronchese and Kern (1953) and Tomaszewski (1953). Pruritus ani as a sequel of an abundance of monilia organisms subsequent to medication with antibiotics is not infrequent. Such cases were reported by Pappenfort and Schnall (1951), Müller and Vogt (1951) and Sidi and Plas-Arditti (1954) and are evidently frequently seen after medication with chlortetracycline, streptomycin and oxytetracycline. Similarly, fissuring of the labia minora and majora and vulvovaginitis due to C. albicans have been reported after medication with certain antibiotics such as chlortetracycline, as reported by Harris (1950) and Pappenfort and Schnall (1951). In view of the report of Liston and Lees (1940), epididymitis may also be caused by a superimposed monilia infection.

It is therefore apparent that suppression of the normal bacterial growth in the intestinal tract

may lead to secondary infections which, although in most instances of less serious nature, may occasionally lead to severe and even fatal complications.

It has also been shown that changes of the bacterial flora in the intestinal tract may lead to deficiency in certain vitamins, which in turn may cause toxic manifestations. Haubold, Loew and Kolb (1953) showed that in normal subjects the intramuscular administration of 800,000 and 1,600,000 U. of penicillin causes, after twenty-four hours, a distinct lowering of the vitamin A level in the serum from 90 and 125 I.U. to 30 and 50 I.U., presumably because of some effect on the vitamin depots. Di Raimondo, Mannino and Trinchese (1952) showed that in rats prolonged oral administration of oxytetracycline, chloramphenicol and chlortetracycline causes a marked reduction of the levels of nicotinamide, folic acid and cobalamin (vitamin B_{12}) in the liver and some reduction of the pyridoxine level, and they suggested that these changes were related to changes in the intestinal bacterial flora resulting in blocking of the vitamin formation in the intestine. Di Raimondo, Anagarano, Mannino and Trinchese (1952) studied in normal subjects and in patients the effect of antibiotics on the urinary excretion of vitamin B complex, folic acid, nicotinamide, pyridoxine and vitamin B_{12} and found that these were markedly reduced after medication with oxytetracycline, chloramphenicol and chlortetracycline at therapeutic levels. In undernourished persons the administration of these antibiotics produced clinical signs of avitaminosis as well as a reduction in the urinary excretion of these substances; these effects were paralleled by the disappearance of intestinal bacteria, especially of the coliform type. Harris (1950) drew attention to the similarity between "furred tongue" of patients under medication with chlortetracycline and the picture of riboflavin deficiency and he considered vaginitis and vulval and anal irritation also a result of such deficiency. This may also be the explanation of the observation of Tomaszewski (1953) who reported twenty-three cases of "black tongue" in patient after medication with penicillin, chlortetracycline, chloramphenicol and streptomycin without evidence of abnormal fungal or bacterial growth. Hartmann and Stabel (1950) noted in a few patients after oral administration of penicillin or penicillin-sulfonamide nutritional and clinical disturbances similar to those seen in pellagra, characterized by slow thinking, mental depression, peripheral paresthesias, reduced sensation, ataxia and attenuated or negative tendon reflexes, symptoms which rapidly disappeared after discontinuance of the drug and medication with nicotinic acid. Similarly, Merliss and Hoffman (1951) assumed, as already pointed out, that steatorrhea observed after medication with certain antibiotics was due to a deficiency state secondary to destruction of the normal intestinal bacterial flora, especially because it responded promptly to the injection of liver extract in conjunction with the oral administration of B vitamins. Sacks, Bradford and Spurling (1951) reported on a case of aplastic anemia after prolonged medication with streptomycin which in their opinion might have been caused by folic acid deficiency because of alterations of the intestinal flora. Escher and Roost (1951) showed that feeding of vitamin A alleviates the nephrotoxic action of the simultaneous administration of streptomycin and uranium nitrate. Humphreys and associates (1953) assumed that changes in the prothrombin time and clotting time produced by certain antibiotics were due to a decrease of vitamin K because of changes in the intestinal flora and the suppression of those organisms which are responsible for the synthesis of this vitamin.

It may be speculated that the prompt bactericidal action of antibiotics and the resulting rapid inhibition of infectious processes may lead to a *reduction of antibody formation* and thus reduce the resistance of the patient to reinfection by the same organism. This possibility seems to be supported by some experiments reported by Slanetz (1953) who found that feeding mice and rats a diet containing 0.1 per cent of aureomycin, oxytetracycline and chloramphenicol for a prolonged period of time interferes with antibody formation against Salmonella enteritidis B27n, whereas feeding of the same diets for a short period of time has the opposite effect. Schmeiser (1953) found no evidence in 6,344 cases of scarlet fever that medication with penicillin augments the number of relapses after an interval of at least 6 weeks or that it interferes with antibody formation, but Foerster and Leopold (1953) presented definite evidence that in scarlet fever penicillin reduces the immune reactions and unfavorably influences the epidemiology of the disease. Smadel (1954) concluded from a study of the immunologic response to antibiotics in patients with scrub

typhus that under certain circumstances specific therapy may delay or diminish antibody production by interfering with the development of the usual quantity of antigen.

It is, therefore, apparent that medication with antibiotics is by no means a harmless matter and that a great variety of complications may arise if they are not used properly and without close control of the condition of the patient.

So far only those dangers have been discussed which have a direct bearing on the health of the patient but there is another imminent danger resulting from the indiscriminate use of antibiotics. This centers around the question as to whether or not the promiscuous use of antibiotics will result in an increasing number of *antibiotic-resistant strains of pathogenic organisms* and thus reduce and finally abolish the efficacy of these valuable therapeutic agents. Whereas Long (1952) stated that from a practical point of view the physician can assume that resistance to penicillin resulting from bacterial mutation or adaptation is rarely of clinical importance, Serck-Hanssen (1952), studying the susceptibility to penicillin in postoperative infective wounds in a hospital, found the great majority of strains of Staph. aureus highly resistant to penicillin and that in some cases the infection was evidently due to resistant strains. In addition, pathogenic staphylococci were found in over 50 per cent of the nursing staff and these were almost all highly resistant to penicillin. Similarly, Eisenberg and associates (1953) found an incidence of 42 per cent of penicillin-resistant strains of staphylococci and streptococci whereas more than 90 per cent were found to be susceptible to erythromycin or carbomycin. Jackson and collaborators (1953) found a significant decrease in sensitivity to penicillin of strains of coagulase-positive hemolytic streptococci which, however, was not the case with other gram-positive bacteria. Lepper and associates (1953) found that in patients treated with penicillin a significant percentage of staphylococci showed increased resistance to this antibiotic. Ludlam (1953) found that infants born in a hospital harbored a much higher proportion of resistant strains of Staph. aureus than those born at home, and the same was found to hold true for staphylococci isolated from the nose of their mothers; further, that patients apparently frequently acquired resistant strains while in a hospital. He believed that there is a definite risk that the population is gradually being infiltrated with resistant strains. A similar report was published by North and Christie (1945) who found a greater incidence of penicillin-resistant strains of staphylococcus in a hospital in which penicillin was used than in another one in which this antibiotic was not used. These findings appear to be supported by a report of Rantz (1954) who stated that in 1943 90 per cent of all strains of Staph. aureus were inhibited by a few tenths of a unit of penicillin per cubic centimeter whereas ten years later about 75 per cent of the strains isolated in clinical laboratories were found to be highly resistant, as reported by Finland and Haight (1953). Similarly, Needham and Nichols (1953) reported that by 1948 the number of penicillin-resistant strains of Micrococcus pyogenes had reached 60 per cent but had not increased thereafter. Welch (1953) reviewed this subject and came to the conclusion that although there has been some increase in the number of penicillin-resistant staphylococci the incidence of such strains is greater in the hospitalized than in the general population.

According to Long (1952), many streptomycin-resistant strains have developed since the introduction of this antibiotic so that in this country its use should be restricted to the treatment of tuberculosis. According to Jackson and coworkers (1953), the incidence of increased resistance of gram-positive and gram-negative bacteria was much greater with streptomycin than with penicillin, and appears to vary markedly corresponding with the widespread or restricted use of this drug. This was also pointed out by Needham and Nichols (1953).

Whereas Long (1952) stated that increased resistance to oxytetracycline appeared to be no pressing problem at that time, subsequent publications indicate that this situation has changed in more recent years. Needham and Nichols (1953) stated that since 1948 the number of oxytetracycline-resistant strains of micrococci has gradually increased, but according to Wright and associates (1953) the resistance is usually of a lower degree than with penicillin, streptomycin and erythromycin. Similarly, Eisenberg and coworkers (1953) found that resistance to staphylococci and streptococci to oxytetracycline is somewhat less frequent than with penicillin. According to Finland and Haight (1953), 33% of 500 strains of Staph. aureus had become resistant to this drug.

Needham and Nichols (1953) found no strains of M. pyogenes in 1948 which were resistant to chlortetracycline but the incidence of resistance to this antibiotic increased gradually to 36 per cent during the subsequent three years. This was also reported by Dearing and Fordyce (1953). Llewellyn (1953) reported that in 1949 all penicillin-resistant strains of M. pyogenes were sensitive to as little as 0.78 μg. of aureomycin but two years later 9 per cent of such strains were resistant to more than 6.2 μg., and after a period of two more years 36 per cent were resistant to this antibiotic. Similar findings were reported by Lepper and associates (1953). According to Finland and Haight (1953), the incidence of chlortetracycline-resistant strains of Staph. aureus is somewhat lower than with oxytetracycline.

Llewellyn (1953) encountered no cases of natural resistance of M. pyogenes to erythromycin but he stated that in two patients treated with this antibiotic an originally sensitive strain had become highly resistant after a short period of time. According to Wright and associates (1953), strains of staphylococci and streptococci may develop resistance to this antibiotic quite readily.

As shown by Needham and Nichols (1953), the incidence of strains of M. pyogenes resistant to chloramphenicol has increased only slightly over the past years, presumably because this drug was used only rarely in the hospitals where this study was made. But Wheeler and Wainerman (1954), treating babies suffering from diarrhea due to Escherichia coli 0-III with chloramphenicol, noted such a marked increase in the number of chloramphenicol-resistant strains that successful treatment with this drug was impossible.

Jackson and coworkers (1953), studying the antibiotic sensitivity of the major gram-positive and gram-negative species of bacteria to bacitracin, noted no signs of increased resistance to this antibiotic.

As indicated by this short review of the literature, the resistance of various microorganisms to antibiotics is increasing in various degrees. Because of lack of comparative data an exact evaluation is difficult to make but, as pointed out by Bondi and associates (1954), the general increase of streptococcal infections is probably related to the increased incidence of antibiotic-resistant strains. Because of this possibility antibiotics should not be incorporated into such articles as chewing gum and toothpaste and their use should be restricted to therapeutic needs.

CONCLUDING REMARKS

The various observations and reports incorporated in this review of complications of antibiotic therapy demonstrate clearly that antibiotics are very valuable but by no means harmless therapeutic agents and that they have to be used with adequate precautions and not indiscriminately. The incidence of severe and fatal complications may not appear alarming in view of the great quantities of antibiotics produced annually but, as pointed out by Pickard and Rosenblatt (1954), in many instances of these toxic reactions medication with antibiotics was not warranted. A sad illustration to the point is one fatality reported by Fisher (1954) which resulted from repeated medication with penicillin. This refers to a healthy young man who had undergone a minor operation on his foot which was followed by "prophylactic" intramuscular injection of 300,000 units of penicillin, whereupon he died within a few hours from anaphylactoid shock. Afterward it was learned that a few months previously he had been treated with penicillin for a minor illness, both measures evidently not being indicated or necessary.

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Seminars on the Hemolytic Anemias

The Auto-Immune Haemolytic Anaemias*

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IT is only within the last ten years that the development of antibodies directed against a patient's own erythrocytes has been recognized as the probable cause of certain types of acquired haemolytic anaemia. Auto-haemagglutination had been observed many years previously, for as long ago as 1908 Widal, Abrami and Brulé[1] stressed that the phenomenon was a characteristic feature of the blood of patients with "l'ictère hémolytique acquis." The significance of the agglutination was, however, overlooked, and it was not until after the antiglobulin test of Coombs, Mourant and Race[2] had been applied to the study of cases of acquired haemolytic anaemia that the concept of an auto-immune type became generally accepted. Now it is known that the majority of patients who develop acquired haemolytic anaemia do so because they form proteins, having the properties of anti-erythrocyte antibodies, which are capable of seriously diminishing the life-span of the patients' own corpuscles. These proteins are referred to as *auto*-antibodies, in contradistinction to *iso*-antibodies, such as the naturally occurring anti-A or anti-B and the immune type of antibody developing as the result of transfusion or pregnancy, which do *not* affect the patients' own corpuscles.

In this short review an attempt will be made to survey briefly the classification and clinical, haematologic and serologic features of the auto-immune haemolytic anaemias, as well as aetiology, pathogenesis and treatment.

CLASSIFICATION

From the clinical standpoint the most important division is (1) into cases of unknown origin (primary or idiopathic cases), the clinical course of which may be transient but is more often chronic, and (2) symptomatic (secondary) cases in which the haemolytic anaemia appears to be a complication of or a sequel to some underlying disease, the most frequent of which seem to be chronic lymphatic leukaemia or reticulosarcoma, disseminated lupus erythematosus and virus pneumonia. The haemolysis is acute and transient in patients with postvirus pneumonia

TABLE I
A CLASSIFICATION OF THE AUTO-IMMUNE HAEMOLYTIC ANAEMIAS

Clinical Types	Aetiology	Serology (Type of Antibody)
Primary (idiopathic) acquired haemolytic anaemias: Transient, Chronic	Unknown	Warm (majority); Cold (minority)
Secondary (symptomatic) acquired haemolytic anaemias		
Transient	Postvirus pneumonia	Cold
	(?) Due to other viruses	(?) Warm or cold
Chronic	Associated with chronic lymphatic leukaemia, reticulosarcoma, disseminated lupus erythematosus, etc.	Warm (majority); Cold (minority)
	Syphilis (some cases of PCH)	Cold

haemolytic anaemia but is usually chronic in the other types of secondary case.

Paroxysmal cold haemoglobinuria (PCH) is also an auto-immune haemolytic anaemia. In its chronic form, often but not apparently invariably associated with syphilis, the clinical syndrome of PCH is, as a rule, quite distinct from that of other types of auto-immune haemolytic anaemia. However, transient forms occur which may be clinically indistinguishable from other types of acute haemolytic anaemia.[3] PCH will not be referred to further in this paper as the subject is being dealt with in a later seminar by Dr. T. Hale Ham.

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From the *aetiologic* standpoint the auto-immune acquired haemolytic anaemias can be divided into a minority in which a viral or postviral origin can be established, as in patients who develop a haemolytic episode following virus pneumonia, and a majority in which the cause is as yet unknown. From the *serologic* point of view they may be classified into two types according to whether the antibodies are "warm" or "cold" ones.

It is thus possible to characterize the auto-immune haemolytic anaemias in at least four ways: (1) according to whether the clinical course is a transient or a chronic one; (2) into primary or secondary cases; (3) into those of known or unknown aetiology or (4) according to two serologic types. In Table 1 is shown an attempt at intercorrelation of these different methods of classification.

CLINICAL FEATURES

Only a brief account of the clinical manifestations of the auto-immune haemolytic anaemias can be attempted. They are not confined to any particular race and there is no obvious genetic basis for the disease. Indeed, one of the author's patients[3] has an identical twin sister who is still unaffected nine years after the onset of the disease in her twin.

Age and Sex. Subjects of all ages are affected, as well as both sexes, but the idiopathic type of the disease, at least, appears to be more common in females.[4] The age distribution of forty-seven patients studied by the author is shown in Figure 1; twenty-five of thirty-seven patients suffering from the idiopathic type of the disease were females. The data assembled in Figure 1 also suggests that the cold-antibody type of the disease particularly affects elderly subjects.

NATURAL HISTORY OF THE DISEASE

I. Idiopathic Cases

Auto-immune acquired haemolytic anaemia is a very variable disorder and almost every grade of severity may be encountered. More often than not the illness is a chronic one, with an insidious onset, pursuing a fairly steady course extending over years. Occasionally, the illness may be characterized by successive acute relapses.[5] In other patients the disorder starts insidiously but, by steadily increasing in severity, brings about the death of the patient.[3] Some patients, however, recover spontaneously after a short-lived episode of acute haemolysis (so-called Lederer's anaemia) or within a few months of an illness commencing insidiously.[3] In the most severely affected patients the haemolysis may be accompanied by haemoglobinuria. Occasionally, symptoms and signs of thrombocytopenic purpura precede or accompany those of acquired haemolytic anaemia.[3,6]

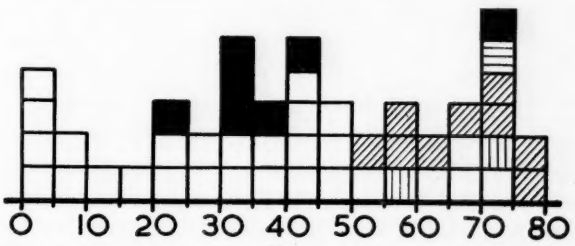

FIG. 1. Age distribution of forty-seven patients suffering from auto-immune haemolytic anaemia. White box, warm antibody type; parallel lines, secondary warm antibody type; cross hatching, secondary cold antibody type; and black box, cold antibody type after virus pneumonia. (Reproduced from "The Haemolytic Anaemias: Congenital and Acquired," Churchill, London.)

In patients in whom the auto-antibodies are of the cold type a rather characteristic clinical syndrome may develop.[3,7,8] Their illness is usually a very chronic one and in most patients is more severe in cold weather; they then show signs of Raynaud's phenomena, with their fingers, ear lobes and tip of the nose becoming cold and blue on exposure to cold. They may also experience haemoglobinuria in particularly cold weather.

Physical Signs. In addition to signs of anaemia the patients are usually slightly to moderately jaundiced. The jaundice is typically acholuric, but in acute crisis the jaundice often deepens and bile may be found in the urine. As already mentioned, purpura may occasionally be met with.

The *spleen* is probably always enlarged and is usually palpable; it rarely, however, extends below the umbilicus. The *liver* may be slightly enlarged, particularly in severely ill patients.

The *urine* usually contains excess urobilin, Haemoglobinuria may develop in severely ill patients with haemolytic anaemia due to warm antibodies[3] and in patients with cold antibodies as the result of exposure to cold. The *faeces* contain excess urobilinogen.

II. Secondary (Symptomatic) Cases

After Virus Pneumonia. This type of haemolytic anaemia, although rare, is now well

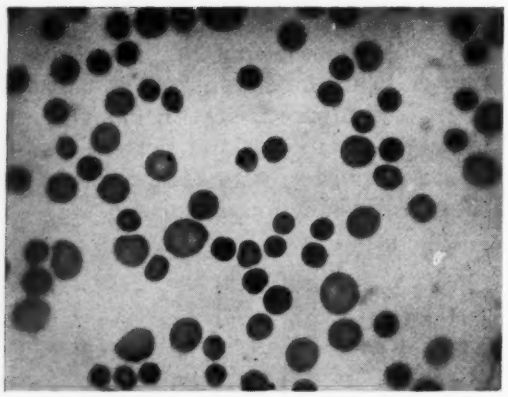

FIG. 2. Photomicrograph of a blood film of a patient with auto-immune haemolytic anaemia of the warm antibody type. There is marked microspherocytosis, × 400.

recognized.[3,9,10] It develops in a small proportion of patients who form cold antibodies after virus pneumonia. The onset is usually sudden and typical signs of haemolysis develop at the end of the second or during the third week of illness, often in fact when the patient is convalescing from his pneumonia. The haemolysis may be serious and is sometimes accompanied by haemoglobinuria. However, spontaneous recovery takes place within a week or so; the prognosis is good and the mortality low.[4]

After Other Virus Infections. The only other virus infection after which haemolytic anaemia has occurred with any frequency is infectious mononucleosis.[11,12,66] Even so haemolytic anaemia is of rare occurrence and the clinical and serologic patterns seem less uniform than in the postvirus pneumonia cases. The role of viruses in haemolytic anaemia is considered again later under "Aetiology."

In Association with Chronic Lymphatic Leukaemia, Reticulosarcoma and Disseminated Lupus Erythematosus (DLE), etc. It is being increasingly realized that auto-immune haemolytic anaemia may not infrequently be symptomatic of some underlying systemic disorder affecting lymphoreticular tissue or may be associated with disorders of obscure pathogenesis such as DLE or periarteritis nodosa.[5,13–17] Indeed, in some patients the haemolytic anaemia has dominated the clinical picture, the underlying accompanying disorder only manifesting itself months or even years subsequently.[3,5]

The incidence of symptomatic cases varies in different published series. Sacks, Workman and Jahn[4] reported that of 166 patients collected from the literature or studied by themselves, eighty-five had developed haemolytic anaemia secondary to some underlying disease; the total included postvirus pneumonia haemolytic anaemias. Ten of forty-nine patients investigated by the author[3] were suffering from symptomatic haemolytic anaemia; seven of these followed virus pneumonia, in two there was an accompanying reticulosarcoma and in one chronic lymphatic leukaemia. Dausset and Malinvaud[17] found that of forty-seven patients with warm antibodies thirty-five had the idiopathic type of the disease and twelve symptomatic haemolytic anaemia associated with various types of reticulosis, reticulosarcoma or lymphatic leukaemia. Crosby[66] reported that of sixty patients whose records were studied by the U.S.A. Armed Forces Institute of Pathology 40 per cent were suffering from the symptomatic form of the disease. He pointed out that the patients affected with haemolytic anaemia associated with malignant lymphoma were predominantly in the older age group, e.g., one patient of sixteen at age twenty to thirty years had a malignant lymphoma compared with nine of seventeen at fifty to sixty years.

HAEMATOLOGY

Erythrocytes. All grades of severity of anaemia may be encountered. Mean corpuscular volume measurements usually reveal macrocytosis. However, inspection of stained films and cell diameter measurements often reveal microcytosis due to the presence of microspherocytes. (Fig. 2.) The spherocytes are usually although not invariably conspicuous whenever haemolysis is active; they are not as a rule found when the disease is completely quiescent. Polychromasia is noticeable in the stained blood films of patients who have high reticulocyte counts, and normoblasts can usually be found in small numbers in the blood of severely anaemic patients. Siderocytes may be present in the peripheral blood of patients in whom haemolysis is occurring at a very rapid rate, even before splenectomy.[3]

Leukocytes. In acute haemolytic episodes the total leukocyte count may exceed 30,000 cells per cu. mm., chiefly due to an increase in neutrophils; small numbers of myelocytes may also be present. In hyperacute cases erythrophagocytosis by monocytes may be seen if freshly made films are carefully examined;[3,18] in buffy-coat preparations of incubated blood the phenomenon may be more frequently found.[19]

In chronic cases of acquired haemolytic anaemia a neutropenia is not infrequent.[3]

Platelets. The platelet count is normal or low; occasionally thrombocytopenia may be marked and accompanied by clinical purpura.[6,20,66]

Osmotic Fragility. There is usually a moderate increase in osmotic fragility which corresponds with the degree of spherocytosis. The increases are found with antibodies of the cold type as well as with antibodies of the warm type. (Fig. 3.) In clinically hyperacute haemolysis the increase in fragility may be so marked and the erythrocytes so spherocytic that some haemolysis occurs even in 0.85 per cent sodium chloride solutions.

The increase in osmotic fragility which follows incubation at 37°C. for twenty-four hours is usually greater than normal, but the increase is less regular than in hereditary spherocytosis. Autohaemolysis following incubation almost invariably exceeds the normal. The increase parallels the degree of spherocytosis present; it may be particularly rapid in patients in acute haemolytic crises.[21] In the patient described by Dacie[3] as Case 12 haemolysis increased so rapidly after blood was withdrawn that it was impossible to obtain unhaemolyzed serum.

Auto-agglutination. The blood of patients with auto-immune haemolytic anaemia commonly undergoes rapid spontaneous auto-agglutination after withdrawal, irrespective of whether it is oxalated or defibrinated. In some cases, those due to cold antibodies, this is due to cold agglutination resulting from the temperature of the blood being allowed to fall to 30°C. or below. (Fig. 3.) In other patients, those with warm antibodies, auto-agglutination may occur even if the blood is kept at 37°C.; this seems to be due to the patient's erythrocytes, if heavily sensitized with incomplete warm antibodies, undergoing agglutination in undiluted plasma or serum.

Serum Bilirubin. The serum-bilirubin concentration is usually found to be between 1 to 4 mg. per 100 ml.; values higher than this suggest that the patient may be suffering from chronic liver disease or, if associated with a haemolytic crisis, from acute liver-cell damage.[22]

Plasma Haemoglobin. Small increases above the normal concentration are commonly found.[23] However, in hyperacute cases with marked spherocytosis or in patients with very high titre cold antibodies the plasma may be visibly red-brown in colour and contain oxyhaemoglobin and methaemalbumin.

PATHOLOGY

Excluding from consideration the pathologic changes resulting from the presence of some underlying disease, certain changes common to all types of auto-immune haemolytic anaemia will be referred to briefly. The *bone-marrow* undergoes hypertrophy and may become predominantly erythropoietic; haemopoiesis is typically normoblastic.

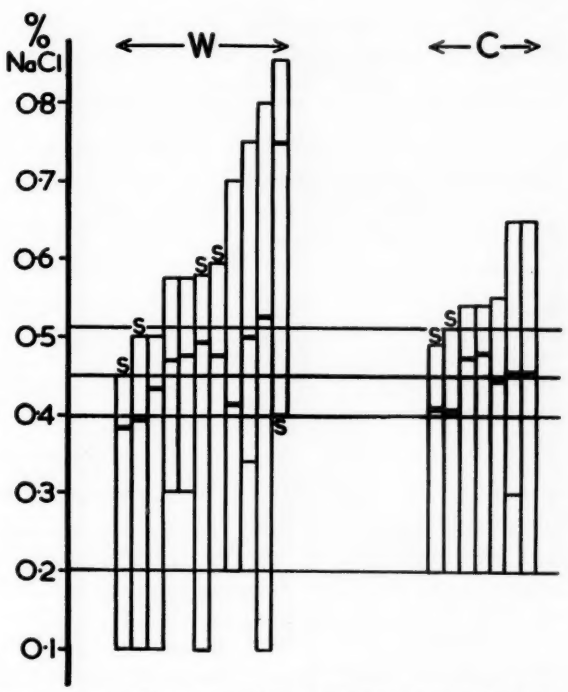

FIG. 3. Results of osmotic fragility determinations in eleven patients with auto-immune haemolytic anaemia of the warm antibody type (W) and in seven patients with auto-immune haemolytic anaemia of the cold antibody type (C). The observations on each patient are represented by an upright rectangle. The horizontal lines indicate the normal range for osmotic fragility and the double lines the normal range of the M.C.F. (median corpuscular fragility). S denotes after splenectomy. (Reproduced from "The Haemolytic Anaemias: Congenital and Acquired," Churchill, London.)

The *spleen* is usually enlarged from twice to five times its normal size. Engorgement with blood, although sometimes intense, is not such a regular feature as in hereditary spherocytosis;[24] the reticulum cells of the spleen pulp are usually hyperplastic and some evidence of erythrophagocytosis may be found. Small foci of myeloid metaplasia are generally present, at least in fatal cases. Siderosis is often intense, particularly in patients who have received frequent transfusions.

The *liver* is usually enlarged in fatal cases and there may be areas of focal necrosis. The Kuppfer cells are often hypertrophied. In patients in whom the plasma-haemoglobin concentration is raised, siderosis of the *kidney* tubules may be conspicuous. In these patients haemosiderin may be identified in the urine deposit by means of the ferrocyanide reaction. The *lymph nodes* are not as a rule significantly enlarged. However, an unusual amount of erythrophagocytosis may be observed in the lymph sinuses.

TABLE II
INCIDENCE OF WARM AND COLD ANTIBODIES IN AUTO-IMMUNE HAEMOLYTIC ANAEMIAS

Type of Auto-immune Haemolytic Anaemia	Type of Antibody	
	Warm	Cold
Idiopathic	30	9
Symptomatic:		
Chronic lymphatic leukaemia	1	0
Reticulosarcoma	1	1
Postvirus pneumonia	0	7

ERYTHROCYTE SURVIVAL STUDIES IN AUTO-IMMUNE HAEMOLYTIC ANAEMIA

Mollison[25] was probably the first worker to demonstrate by means of Ashby's method of differential agglutination that normal erythrocytes transfused to a patient with acquired haemolytic anaemia were rapidly destroyed in the recipient. Brown, Hayward, Powell and Witts[26] found that if the number of surviving normal corpuscles was plotted against time, the curve of elimination formed a curve, being at first rapid and then becoming progressively less rapid. They suggested that the curved graph of elimination meant that an exponential haemolytic mechanism was at work which resulted in the destruction of the erythrocytes irrespective of their age.

Erythrocyte survival studies by the Ashby technic have subsequently been widely used to assess the rate of haemolysis and also in diagnosis, the survival of transfused normal erythrocytes in the congenital haemolytic anaemias, due to intrinsic inherited erythrocyte defects, being characteristically normal.

The recent introduction of the radioactive chromium (^{51}Cr) technic for studying erythrocyte survival has provided another useful tool in investigation.[27–29,67] With this method it is possible to tag the patient's own cells and reinject them into him. One of the weaknesses of the Ashby method was that it did not allow the patient's own cells to be studied in his own circulation, and it had to be assumed (without any evidence for or against) that study of the survival of normal cells gave a good indication of what was happening to the patient's own erythrocytes. The chromium technic allows a direct comparison to be made between the survival of the patient's cells and normal cells. Preliminary studies suggest that the patient's own cells may survive better than studies with normal cells have indicated.[30]

SEROLOGY OF AUTO-IMMUNE HAEMOLYTIC ANAEMIAS

The auto-antibodies of acquired haemolytic anaemia conform to two main types: *warm* antibodies acting *in vitro* at 37°C. at least as well as at lower temperatures, and *cold* antibodies, the activity of which is markedly potentiated by cold. They are inactive or only weakly active at 37°C. The warm type of antibody appears to be most frequently encountered. The distribution of the two types of antibody in the patients investigated by the author is shown in Table II.

Specificity of the Antibodies. As well as being auto-antibodies, the antibodies of acquired haemolytic anaemia act as iso-antibodies and affect normal human corpuscles. It used to be thought that they reacted with normal corpuscles irrespective of blood group or type, i.e., that they were always "non-specific" antibodies. However, recent work has shown that some sera contain components reacting specifically with identifiable blood-group antigens; in almost all instances these have been within the Rh blood-group system.[31–35] In rare instances no non-specific component can be identified, e.g., Dacie's Case 12,[3] whose antibody was a mixture of anti-e and anti-C, the patient's own Rh genotype being CDe/cde. Cold antibodies on the other hand seem, in most instances at least, to be "non-specific" in type.[3]

Recognition of Erythrocyte Sensitization. The presence of antibody adsorbed to the patient's erythrocytes is best detected by the antiglobulin test of Coombs, Mourant and Race.[2] The reaction is always positive, with possible very rare exceptions, in auto-immune haemolytic anaemia of both the warm and cold antibody types. It is essential to use the antiglobulin serum at the

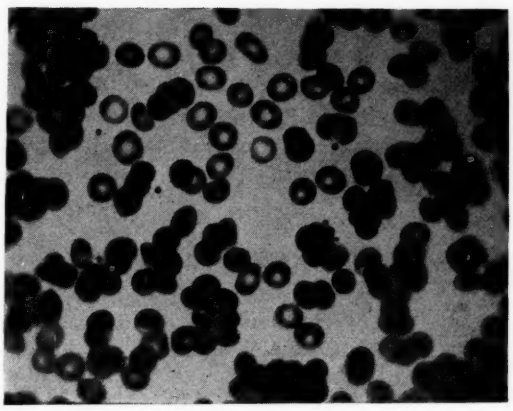

FIG. 4. Photomicrograph of a peripheral blood film of a patient with auto-immune haemolytic anaemia of the cold antibody type. There is conspicuous autohaemagglutination, × 400.

optimum dilution for the type of antibody under investigation. However, it must not be assumed that a positive result necessarily indicates that the patient is suffering from active haemolytic anaemia. Quite strongly positive results may be obtained with the corpuscles of patients in clinically complete remission following splenectomy as well as rarely in apparently completely healthy subjects. Weakly positive tests may also sometimes be given by the blood of patients suffering from a variety of disorders affecting protein formation at a time when haemolysis, although possibly latent, is not clinically obvious, e.g., in rheumatoid arthritis, sarcoid and disseminated lupus erythematosus.

DETECTION OF ANTIBODIES IN PATIENTS' SERA

Warm Antibody Type. Antibody can generally be detected in the serum of patients in whom haemolysis is proceeding actively by means of the indirect antiglobulin test or by the use of trypsinized erythrocytes. In patients in clinical remission, on the other hand, the indirect antiglobulin test is usually negative, although components of antibody may still be present which react with trypsinized cells.[3] "Non-specific" cold agglutinins may be present in normal or slightly raised concentrations; the titre rarely exceeds 512 at 2°C. (Fig. 4.)

Cold Antibody Type. Antibody can probably be invariably found free in the patient's serum in high concentrations even if the patients are but mildly affected clinically. This is because cold antibodies are relatively poorly adsorbed at temperatures near body temperature. At 2°C. the cold agglutinin titre using normal corpuscles may be very high, even reaching 256,000. (Fig. 5.) It is characteristic of the cold antibodies of acquired haemolytic anaemia that quite high agglutinin titres are obtained at 20°C. and that agglutination may even occur at temperatures as high as 30°C. These high titre cold antibodies are also potentially haemolytic;[36]

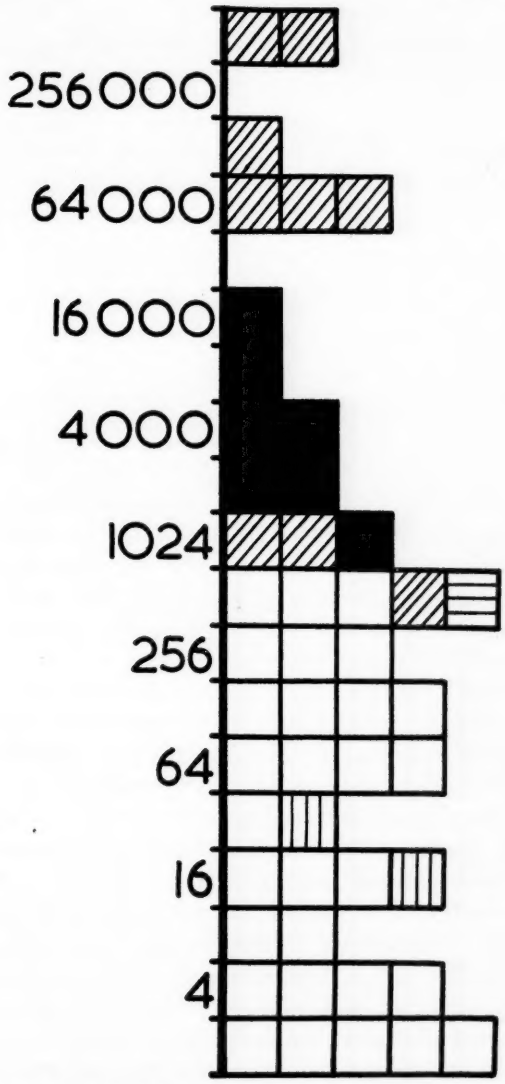

FIG. 5. Cold agglutinin titres at 2°C. obtained from the sera of forty-eight patients suffering from auto-immune haemolytic anaemia. Symbols are the same as those in Figure 1. (Reproduced from "The Haemolytic Anaemias: Congenital and Acquired," Churchill, London.)

they cause lysis of normal corpuscles in low serum dilutions—if the pH of the serum-corpuscles suspension is adjusted to the optimum, usually pH 6.5 to 7.0—and lysis of the very sensitive paroxysmal nocturnal haemoglobinuria (P.N.H.) erythrocytes in very high serum dilutions. Incomplete cold antibodies are usually

found to be associated with the agglutinating and haemolytic antibodies, in most instances in comparable titres. The thermal range of the incomplete antibodies seems often to be higher than that of the agglutinating or haemolytic antibodies, for in eight of sixteen cases of auto-immune haemolytic anaemia of the cold antibody type investigated by the writer the indirect antiglobulin reaction was positive even if the sensitizations were strictly carried out at 37°C. This finding is clearly related to the apparently invariable occurrence of positive direct antiglobulin tests in patients with clinically obvious haemolytic anaemia of the cold antibody type.

TABLE III

DIFFERENTIATION OF WARM AND COLD AUTO-ANTIBODIES BY THE ANTIGLOBULIN REACTION

Type of Antibody	Optimum Temperature for Sensitization	Effect of Acidification of the Patient's Serum to pH 6.5–7.0	Inhibition Due to Heating Patient's Serum at 56°C. for 30 minutes	Concentration of Potent Antiglobulin Serum Giving Maximum Agglutination	Inhibition Caused by Adding γ Globulin to Antiglobulin Serum
Warm....	37°C.	Little	Little	Low (1/16–1/256) (zoning reaction)	Usually marked
Cold.....	<37°C.	Usually increases sensitization	Complete	High (1/1–1/4)	Little

Differentiation of Cold and Warm Antibodies. This distinction can be readily made: (1) by making dilutions of the patient's serum in saline and titrating it, using normal and trypsinized normal corpuscles at 37°C. and at 20°C., and (2) by carrying out indirect antiglobulin reactions using normal corpuscles and sensitizing at 37°C. and 20°C. With cold antibodies, not only will the antiglobulin reaction be greatly intensified if the sensitizations are carried out at 20°C. compared with those at 37°C., but it will be completely abolished if the patient's serum is previously inactivated by heating at 56°C. for thirty minutes. With cold antibodies, too, the degree of sensitization is often increased if the patient's serum is acidified to pH 6.5 to 7.0. With warm antibodies, the degree of sensitization will be at least as great at 37°C. as at 20°C., and acidification of the patient's serum does not as a rule significantly increase sensitization; heat-inactivation of serum does not inhibit sensitization.[38] (Table III.)

Differentiation of the two types of antibody can also often be made from the results of the *direct* antiglobulin reaction. With *warm* antibodies, using potent antiglobulin serum, the maximum reactions are usually obtained using relatively highly diluted antiglobulin serum (e.g., dilutions of 1:32 to 1:256) and the reaction is as a rule easily inhibited by the addition of small amounts of γ globulin to the antiglobulin serum (γ-globulin type of reaction).[37,38] With *cold* antibodies, on the other hand, the strongest reactions are obtained in highly concentrated antiglobulin serum and the reaction is not readily inhibited by adding γ globulin to the antiglobulin serum. (Table IV.)

Serial Antibody Estimations. In following the results of therapy several simple methods are available: (1) quantitative direct antiglobulin reactions using twofold or fourfold dilutions of antiglobulin serum, (2) "auto-agglutinin" titrations using patient's erythrocytes and patient's serum diluted in 20 per cent albumin;[39,40] the results obtained with this test reflect the intensity of sensitization of the patient's corpuscles, their agglutinability in serum-albumin and the amount of free antibody in the serum, and (3) titrations in saline of the patient's serum for "free" antibody, using trypsinized normal corpuscles at 37°C. or normal corpuscles at 2°C. and at 20°C.

OTHER SEROLOGIC FINDINGS

In many cases auto-antibody formation is accompanied by other signs of altered protein metabolism. The total plasma-globulin concentration is frequently raised and abnormal electrophoretic patterns and precipitation tests may be observed.[5] "False positive" tests for syphilis are found in certain patients[3,41,42] and low serum complement levels have been re-

ported; in most instances the associated antibodies have been cold ones.[43,44] Immune iso-antibodies are not uncommonly found in patients who have received transfusions. In the writer's experience anti-E has been most frequently found (five cases). It is possible that patients with auto-immune haemolytic anaemia form iso-antibodies unusually readily.[45]

TABLE IV

CHARACTERISTIC REACTIONS TO ANTIGLOBULIN SERUM OF ERYTHROCYTES FROM PATIENTS SUFFERING FROM AUTO-IMMUNE HAEMOLYTIC ANAEMIA (a) OF THE WARM ANTIBODY TYPE AND (b) OF THE COLD ANTIBODY TYPE

	Dilutions of Antiglobulin Serum						
	1/4	1/16	1/64	1/256	1/1024	1/4096	Control (saline)
(a) Warm antibody type	+	++	+++	++	+	±	0
(b) Cold antibody type	++	+	±	0	0	0	0
	Dilutions of 4% γ Globulin Added to Antiglobulin Serum (1/4)						
	1/4	1/16	1/64	1/256	1/1024	1/4096	Control (saline)
(a) Warm antibody type	0	0	0	0	±	++	+++
(b) Cold antibody type	0	±	++	++	++	++	++

+++ denotes strong agglutination; ± weak agglutination; + and ++ intermediate degrees of agglutination; 0 denotes no agglutination.

AETIOLOGY OF AUTO-IMMUNE HAEMOLYTIC ANAEMIA

The cause of the formation of auto-antibodies is far from clear. Two main mechanisms have been suggested: (1) an alteration in the patient's erythrocytes which has the effect of making them seem foreign to his own antibody-forming mechanism, and (2) the development of anti-erythrocyte antibodies in the course of the formation of abnormal plasma proteins by the patient, the primary abnormality being an unusual reaction on the part of his antibody-forming tissue, rather than a change in his erythrocytes.[46] Although the first hypothesis is attractive there is little real evidence in favour of it, at least as regards the spontaneously occurring disease in man. Experimentally, although it has proved possible in some instances to render the erythrocytes of experimental animals apparently antigenic, haemolytic anaemia has not developed.[47,48] It is possible to put forward more arguments in favour of abnormal protein formation as the fundamental disorder. For example, the increases in total serum globulins, and the positive Wassermann and Kahn and similar reactions which are not uncommonly found fit in with this hypothesis. The association of haemolytic anaemia with neoplasia of the antibody-forming tissue can also be accounted for on this basis. The exact significance of the occurrence of haemolytic anaemia in association with disseminated lupus erythematosus is obscure; possibly the syndrome depends upon the formation of several different types of antibodies.

The role of viruses in the causation of acquired haemolytic anaemia is a matter of controversy. Moolten and co-workers[49–51] have claimed that the virus of Newcastle disease is an important aetiologic factor but their hypothesis has received no support from the work of Morgan[52] and Eyquem and Dausset.[53] The observation of Betke, Richarz and Vivell[54] and of Vivell[68] on two children with acute episodes of haemolytic anaemia suggest that the Coxsackie virus-A may possibly be implicated in some instances. The virus of virus pneumonia is certainly important but here it seems as if it acts as a heterospecific stimulus for abnormal antibody-protein formation rather than upon the erythrocytes directly. It is possible that "idiopathic" cases of acquired haemolytic anaemia similarly develop as a consequence of as yet unrecognized heterospecific stimuli. Even so, an unusual propensity to form antibodies has

probably to be postulated in addition. Finally, why the mechanism which normally prevents the formation of *auto*-antibodies fails to operate remains to be determined.

PATHOGENESIS

The exact mechanisms by which antibodies bring about erythrocyte destructive *in vivo* are not entirely understood. Three effects of antibody action are probably important: (1) *auto-agglutination*, (2) *spherocytosis* and (3) *erythrophagocytosis*.

Erythrocytes highly sensitized by warm antibodies undergo *auto-agglutination* when suspended in serum or plasma. It is possible that this is particularly important in organs such as the spleen where the circulation is slow. Auto-agglutination within the spleen might be expected to result in almost complete arrest of the circulation and an opportunity for further sensitization.[55,56]

Spherocytosis is not readily produced by antibody action *in vitro*. However, *in vivo*, it is a regular accompaniment of experimental antibody-produced haemolytic anaemia in animals, and it is often observed in the spontaneous disease in man. It is a prehaemolytic change and indicates serious damage to the erythrocytes. Castle, Ham and Shen[57] suggested that spherocytosis *in vivo* is a consequence of auto-agglutination causing arrest of the circulation and liberation of injurious metabolites from ischaemic tissue. On the other hand, it is possible that the antibodies adsorbed on to the cell surfaces so affect the erythrocyte metabolism as to cause irreversible damage and that the spherocytosis is the consequence of, and a sign of, this damage.

Erythrophagocytosis in the spleen, liver and lymph nodes and bone marrow appears to be an important mechanism by which cells which have been damaged by antibodies are removed from the circulation.

Cold antibodies probably bring about haemolysis *in vivo* in much the same way as do warm antibodies. However, the antibodies are only important if their thermal maximum is such that agglutination or sensitization can occur at or near body temperature. As already mentioned, sensitization to antiglobulin serum can often be demonstrated to take place *in vitro* at 37°C., and this is probably the cause of the continuous haemolysis from which the patients suffer. In cold weather actual massive auto-agglutination takes place in the peripheral vessels and this in itself leads to further haemolysis and even to haemoglobinuria. The ability of this type of antibody to promote lysis by complement may be an additional cause of haemolysis *in vivo*.

Role of the Spleen. Both clinical and experimental observations indicate that the spleen produces haemolysis in two main ways: (1) by being an organ which filters off sensitized cells from the circulation and (2) by taking part in the formation of auto-antibodies. The clinical effect of splenectomy is discussed below.

TREATMENT

The treatment of acquired haemolytic anaemia of the auto-antibody type is still largely empirical. Until recently there were only two major lines of therapy—blood transfusion and splenectomy. Now ACTH and cortisone provide a third and often potent form of treatment.

Blood Transfusion. In the great majority of patients transfusion cannot be expected to be more than palliative for its value is severely limited by the fact that the survival of the normal blood in the patient is likely to be no better than that of the patient's own erythrocytes.[58] Very seriously ill patients may actually appear worse after transfusion, for the benefit due to a rise in haemoglobin will be transient and the transfusion, by providing him with more erythrocytes to destroy rapidly, will result in an increase in jaundice. Transfusion, nevertheless, may be useful as a preparation for splenectomy, and in obscure cases a determination of the survival of normal transfused blood or of the patient's own cells by the radiochromium technic may help in diagnosis. Great care must be taken in cross-matching the blood to be transfused. An attempt should be made first to determine the specificity, if any, of the patient's antibodies. If non-specific antibodies are present in the patient's serum, blood of the same ABO and Rh group should be chosen which appears *in vitro* to be the least sensitive to the antibodies, as judged by the indirect antiglobulin test.

As the presence of antibodies in the patient's serum may be a cause of reactions the blood should be administered unusually slowly. "Plasma reactions" can be avoided by the transfusion of saline-washed erythrocytes.[59]

Splenectomy. A proportion of patients with acquired haemolytic anaemia benefit from

splenectomy. According to Welch and Dameshek[60] the operation is followed by a complete remission in about 50 per cent of patients. Unfortunately, it does not seem possible to predict when the operation will fail, either on clinical, haematologic or serologic grounds. The amounts of antibody formed—and the proportion of antibody formed by the spleen—and the degree to which the antibody causes auto-agglutination *in vivo* and thus trapping of the erythrocytes in the spleen pulp, are probably important factors in determining the success or failure of the operation. When overwhelming amounts of antibody are being formed, the removal of a part source of the antibodies is unlikely to have any markedly favourable clinical effect. Successful results may be anticipated in both the cold as well as the warm antibody varieties of the disease.[3] Not infrequently, however, after a favourable response lasting days, weeks or even years, the patient relapses and becomes as seriously ill as he was before splenectomy. In patients who do well after splenectomy a diminution in the rate of antibody formation is suggested by the frequent absence of antibody in the patient's serum despite the fact that his erythrocytes usually remain sensitized.[3]

ACTH and Cortisone. The results so far obtained by treatment with ACTH and cortisone are moderately encouraging.[61,62] The majority of patients seem to derive benefit but the results are unpredictable and it is far from clear how the beneficial effect of treatment is brought about. Improvement may be looked for in both the warm and cold types of acquired haemolytic anaemia and in secondary as well as in idiopathic cases.

In some of the patients who respond there seems to be a reduction in the concentration of abnormal antibodies in the serum and in the strength of the direct antiglobulin test, and, exceptionally, auto-antibodies cease to be demonstrable. In other patients the direct antiglobulin test remains strongly positive despite clinical improvement; this has been the author's experience. ACTH and cortisone may prove effective even when splenectomy has failed to be of benefit.[63] If one of the drugs fails to initiate a remission, the other should be tried; for although in most patients cortisone and ACTH appear to be interchangeable, this is not always the case.[16]

PRACTICAL MANAGEMENT OF ACQUIRED HAEMOLYTIC ANAEMIA OF THE AUTO-ANTIBODY TYPE

The outlook in acquired haemolytic anaemia of the auto-antibody type has been substantially altered by the advent of ACTH and cortisone and there is now little doubt that hormone therapy is the initial treatment of choice for any patient who is seriously ill. However, large doses may have to be given, e.g., in adults up to 300 mg. per day of cortisone orally, or up to 200 mg. of ACTH or the equivalent as ACTH gel in divided intramuscular doses. If and when the patient responds, the dosage should be cut down to the minimum which keeps him in remission so as to avoid side effects. There seems no point in giving very large doses in an attempt to obtain a normal haemoglobin concentration for the hormones are probably in no sense a cure for the disease. The best that can be hoped for is to control the severity of the haemolysis and to maintain the patient in fair health until spontaneous recovery takes place. This may necessitate continuing therapy for many months or possibly even years.

Despite the unpredictable result of the operation, splenectomy should be seriously considered in any patient to whom large doses of ACTH and cortisone have to be given for long periods of time in order to obtain a favourab e effect, unless the patients be very young or elderly. In secondary cases splenectomy is less likely to be of lasting value, but good results have been reported particularly in giant follicle lymphoma and in chronic lymphatic leukaemia with haemolytic anaemia.[64,65] The haemolytic anaemia following virus pneumonia and possibly other virus infections is essentially short-lived and no treatment other than keeping the patient warm in bed is usually required. However, if the patient becomes seriously anaemic, he should be given ACTH or cortisone and, if necessary, transfusions.

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Case Reports

Cerebral Mucormycosis: Pathogenesis of the Disease*

Description of the Fungus, Rhizopus Oryzae, Isolated from a Fatal Case

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THIS report concerns an unusual and generally fatal complication of diabetes mellitus which has been described under the name of "mucormycosis." We have recently observed two patients with this fungus infection involving chiefly the central nervous system. In the first case the diagnosis was made on histologic examination only but served to acquaint us with the clinical manifestations of the disease. In our second patient the disorder was suspected before autopsy and appropriate cultures and tissue samples were obtained. The phycomycete Rhizopus oryzae was thus isolated from the affected tissues. As far as we know, this is the first time that the diagnosis of human cerebral mucormycosis has been confirmed by isolation and identification of the etiologic agent.

The term "mucormycosis" is well established in the medical literature but is correct only if applied to infections by members of the order Mucorales as well as to species of the genus Mucor. The order Mucorales encompasses such genera as Absidia, Rhizopus and others which have been implicated in visceral and pulmonary infections. With this reservation the term is acceptable.

The first indication of the pathogenicity of the Mucorales came from Lichtheim's experimental studies in rabbits.[1] Occasional reports of animal and rarely of human infection by these fungi have since appeared in the literature. Infection of the central nervous system has been described only in man.

The clinical features of cerebral mucormycosis are strikingly consistent and are characterized by uncontrolled diabetes with ophthalmoplegia and meningo-encephalitis. At autopsy, invasion of the meninges, brain, cerebral vessels, orbits and paranasal sinuses by the fungus is usually encountered.

CASE REPORTS

CASE 1. A twenty-five year old white housewife was admitted to Emory University Hospital on July 21, 1953, with the chief complaint of dyspnea and weakness. She had been a known diabetic since the age of three, requiring daily doses of 15 to 30 units of protamin-zinc insulin. There had been one definite episode of acidosis several years before admission. Occasional slight elevation of blood pressure was recorded. There had been few systemic or cutaneous infections. For a year preceding admission she had had a dry, hacking cough. Two weeks prior to admission the patient became lethargic, felt "feverish" and noted generalized aching and pain in the right leg. Two days later painful erythematous patches appeared over the right knee and dorsum of the right foot. These lesions soon became papular and increased in size. One week before admission frontal headaches occurred and were followed by dyspnea and polyuria. Headache was usually associated with nasal stuffiness and

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sneezing. Three days prior to admission transient swelling of the right eyelids was noted.

On physical examination the temperature was 99.2°F., the pulse 140 beats per minute, respirations 36 per minute and of Kussmaul type. The blood pressure was 180 mm. Hg systolic and 90 mm. Hg diastolic. The patient was slightly obese, drowsy, but well oriented. The skin was cool and dry. Irregular erythematous, faintly bluish, warm, raised and tender lesions, varying in size from 0.5 to 2 cm. in diameter, were present over both lower extremities. The eyelids, conjunctivas, scleras, pupillary reflexes and ocular fundi were normal except for a slight increase in the arteriolar light reflex. The neck was supple. The lungs were clear except for coarse breath sounds over the right base posteriorly. The liver edge was palpable 2 cm. below the right costal margin. Pelvic examination revealed severe vulvovaginitis with a whitish, creamy, vaginal discharge. Urinalysis showed an acid urine with a specific gravity of 1.018, 3 plus albuminuria, 4 plus glycosuria, 1 plus acetonuria, 170 to 180 erythrocytes and 4 to 6 leukocytes per high power field as well as a rare cellular or granular cast. Urine culture yielded a coagulase-negative staphylococcus and enterococcus but was negative for "pathogenic" fungi. The white blood cell count was 35,500 per cu. mm. with a marked shift to the left. The blood non-protein nitrogen was 49 mg. per cent. The serum sodium was 124.7, chloride 90, potassium 4.5 and carbon dioxide combining power 5.2 mEq./L. The blood sugar on admission was 308 mg. per cent and 265 mg. per cent on the fifth hospital day.

The patient's acidosis responded rapidly to intravenous fluids and a total dose of 250 units of regular insulin. Hypokalemia was treated by oral and intravenous potassium but the serum potassium value remained slightly below normal until the third hospital day when it reached 4 mEq./L. With improved hydration the temperature rose to 102°F. and fluctuated between 101° and 105° throughout the patient's hospital stay. She received intramuscular doses of 600,000 units of penicillin every six hours and 0.5 gm. of streptomycin every twelve hours until the fourth day when the dose of penicillin was increased to 54 million units per day, 30 million of which were given intravenously. The vaginitis cleared after a few days' treatment with alkaline compresses and douches. The skin lesions on the legs, which were believed to represent erythema nodosum, also disappeared. The patient at first became more alert as the acidosis responded to treatment, but she continued to complain of severe headache which was partly relieved by codeine. On the second hospital day left periorbital edema appeared but receded after twenty-four hours. Ophthalmoscopic examination at that time revealed a fixed left pupil, marked retinal edema, thread-like arterioles and stagnating blood columns in arterioles and veins. These findings were interpreted as retinal artery and vein obstruction secondary to infection in the apex of the orbit. Roentgenograms of the sinuses and rhinoscopy were negative. Initial subjective improvement then gave way to progressive lethargy, delirium and disorientation. Leukocytosis, 3 to 4 plus glycosuria and 1 to 3 plus albuminuria persisted. The urine remained acetone-free on a regimen of 20 units of NPH insulin daily, supplemented from time to time by 10 to 20 units of regular insulin. On the fourth hospital day the serum sodium, chloride, potassium and carbon dioxide combining power were normal. The serum phosphorus was 4.5 mg. per cent and calcium 7.3 mg. per cent. The blood non-protein nitrogen did not change. Coma nevertheless became deeper and muscular twitchings, hyperactive reflexes and, terminally, laryngeal stridor developed and the patient died on the seventh hospital day.

Autopsy findings revealed the following: The body was that of a well developed and well nourished white woman. The principal gross findings involved the brain which weighed 1,340 gm. The dura was slightly congested and the dural sinuses were patent. The leptomeninges at the base of the brain and over the inferior surface of the frontal lobes were thickened and adherent to the dura in the region of the left superior orbital fissure. The spinal fluid was grey, turbid and pooled between the flattened convolutions. The inferior portions of both frontal lobes and the tips of both temporal lobes were friable. The cut surfaces showed necrosis, extending from the surface to the left lateral ventricle and virtually destroying the left basal ganglia. No abscess cavities or gross pus were found. The major cerebral arteries showed moderate atherosclerosis. Their patency was not specifically investigated. The posterior half of the cerebral hemispheres, the pons, medulla and cerebellum showed mild edema. Grossly, no areas of infarction were noted. The pituitary, spinal cord, middle ears and paranasal sinuses were grossly normal. The orbits were not

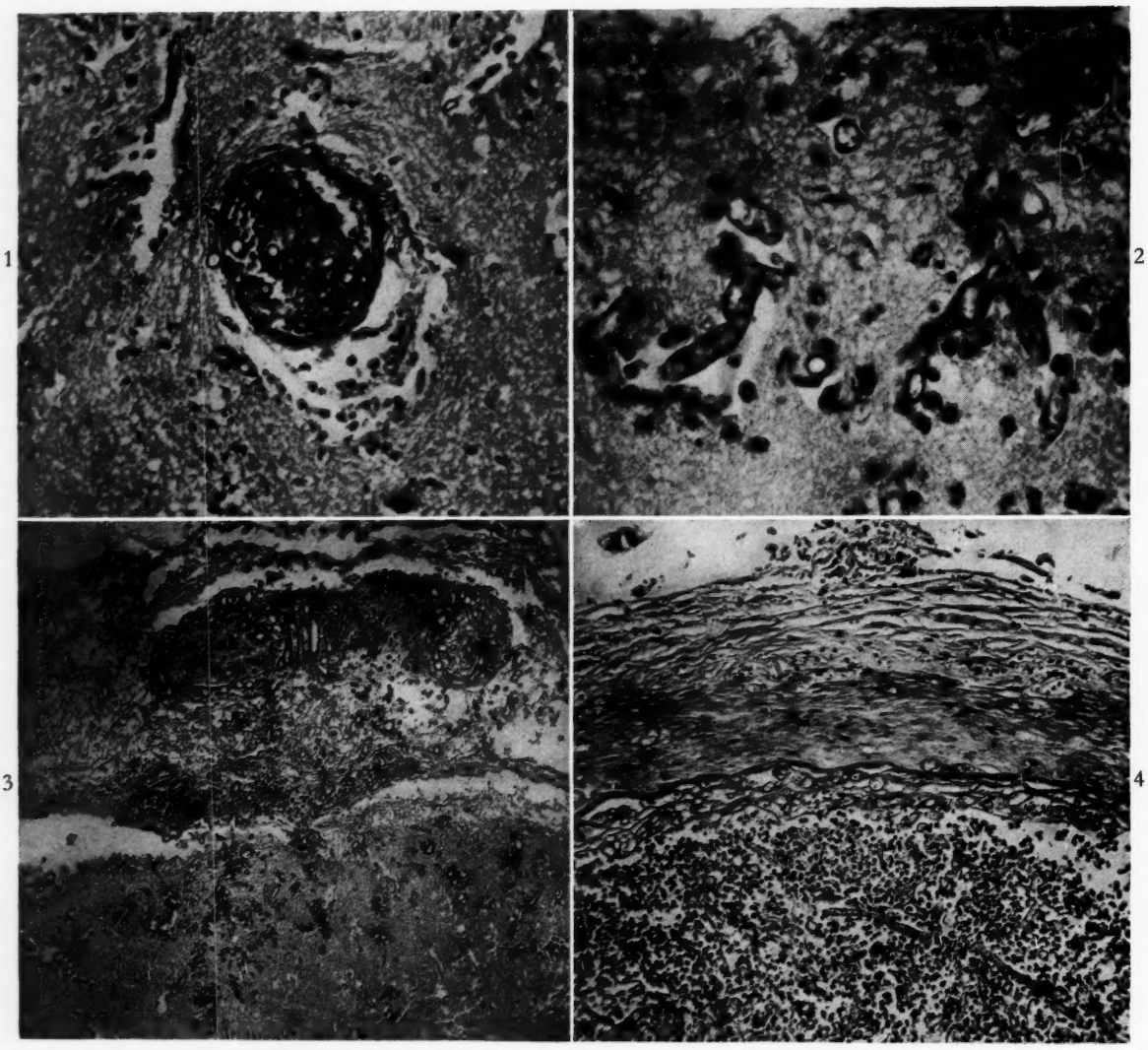

FIG. 1. Medium sized cerebral vessel with thrombus. Note numerous mycelium in thrombus, vessel wall and surrounding tissues; hematoxylin-phloxine; × 220.

FIG. 2. Mycelium in cerebral cortex. Note the large size, aseptate and branching character of the fungus; hematoxylin-phloxine; × 480.

FIG. 3. The meninges, their vessels and the cerebral cortex show heavy infiltration by hyphae with attending inflammatory cell reaction. Note thrombosis of meningeal vessel; hematoxylin-phloxine; × 95.

FIG. 4. Right internal carotid artery with mural thrombus. Many hyphae are present throughout the vessel wall and in the thrombus; hematoxylin-phloxine; × 110.

examined. Additional gross autopsy findings included arteriolar nephrosclerosis and renal papillary necrosis, atrophy of the pancreas (50 gm.), fatty metamorphosis of the liver, atherosclerosis of the coronary arteries and abdominal aorta, acute splenitis, pulmonary congestion and edema.

Sections from the grossly involved portions of the brain revealed extensive necrosis with diffuse infiltration by neutrophilic polymorphonuclear leukocytes and "gitter" cells. Throughout these areas broad, branching, aseptate hyphae, measuring from 6 to 15 μ in diameter and up to 200 μ in length were seen sometimes singly or, often, in clusters. The tubular structure of the hyphae was clearly visible in periodic acid-Schiff preparations. The fungus appeared to grow most luxuriantly in and around blood vessels, infiltrating their walls. The arteries and veins of the brain as well as those of the meninges were involved. The invasion of the vessels by the fungus produced thrombi. (Fig. 1.) While mycelial clusters were attended by inflammatory cells, single hyphae were usually unaccompanied

by such reaction. A section through the pons revealed an area of early necrosis showing an occasional mycelial filament within a small blood vessel. A few neutrophilic polymorphonuclear leukocytes were present at the periphery of the lesion. The diaphragma sellae (turcicae) revealed acute inflammatory cell infiltration but the hypophysis itself was uninvolved. No fungus elements were detected in that area or in sections of other organs. Additional histologic findings were chronic bronchitis, toxic splenitis, intercapillary glomerulosclerosis and renal papillary necrosis. The kidneys showed marked arteriolar sclerosis but no evidence of widespread pyelonephritis. No sections from the paranasal sinuses and orbits were available.

Anatomic diagnoses: Diabetes mellitus (clinical); mucormycosis involving brain and meninges; intercapillary glomerulosclerosis, arteriolar nephrosclerosis and renal papillary necrosis; atrophy of pancreas (50 gm.); fatty metamorphosis of liver; atherosclerosis of coronary arteries and abdominal aorta; chronic bronchitis; toxic splenitis; pulmonary edema and congestion.

Cultures of heart blood, lung and spinal fluid were taken at autopsy. The spinal fluid yielded a yeast-like fungus. This was subcultured on corn meal agar medium but did not form chlamydospores and was unfortunately discarded without further investigation. The other cultures showed no growth.

CASE II. A forty year old Negro was admitted to Grady Memorial Hospital on June 4, 1954. The patient had a three- to four-week history of polyuria, polydipsia, polyphagia and weight loss. On the day preceding admission vomiting, abdominal cramps, progressive lethargy and disorientation developed. There was no past or family history of diabetes and a urinalysis recorded in the outpatient department in 1952 was negative.

Physical examination revealed the following: temperature 94°F., pulse 120 beats per minute, respirations 40 per minute and of Kussmaul type. Blood pressure was 80 mm. Hg systolic and 70 mm. Hg diastolic. The patient was poorly nourished, disoriented and thrashing about. The skin was dry. The fundi showed slight arteriolar narrowing. The deep tendon reflexes were normal bilaterally. Heart, lungs and abdomen were not remarkable except for a few coarse rales in both lungs. The blood hemoglobin content was 13.4 gm. per cent and the white blood cell count was 23,300 per cu. mm. with a marked shift to the left. Urinalysis revealed a specific gravity of 1.018, 1 plus albuminuria, 4 plus glycosuria, 2 plus acetonuria, and 4 to 6 white blood cells per high power field. A urine culture yielded rare yeast-like colonies which were not further identified. The carbon dioxide combining power was 8.1 mEq./L., the blood sugar 370 mg. per cent and the blood urea nitrogen was 29 mg. per cent. During the first twenty-four hours the patient received a total of 6,500 cc. of intravenous fluids including dextran, normal saline and 5 per cent glucose solution. A total of 320 units of regular insulin was administered and the urine became acetone-free after several hours. The sensorium cleared slightly. Laboratory examinations on the second hospital day showed a carbon dioxide combining power of 15 mEq./L., blood urea nitrogen of 43 mg. per cent, blood sugar of 400 mg. per cent and a white blood cell count of 10,350 per cu. mm. The urine remained acetone-free thereafter but a 1 plus albuminuria and 1 to 3 plus glycosuria persisted until the patient's death. Slight clinical hypokalemia was easily corrected by oral administration of potassium. After hydration the temperature rose to about 103°F. and remained at that level until death. The daily urine output rose gradually from 300 cc. during the first day to 1,700 cc. on the day prior to death. The specific gravity dropped to 1.015 on the second and 1.010 on the last hospital day. Smears of the urinary sediment showed yeast forms which were not further studied. On the fourth hospital day complete right ophthalmoplegia was noted but bilateral funduscopy was negative. Because of some nuchal rigidity a lumbar puncture was performed which yielded clear fluid under normal pressure. The fluid revealed a total protein of 50 mg. per cent and contained 16 lymphocytes per cu. mm. Smears and culture were negative. The blood sugar was now 450 mg. per cent. Roentgenograms of the sinuses showed cloudiness of the ethmoid sinuses and the right antrum. On the next day some haziness of the right fundus was noted. The patient had been receiving intramuscular antibiotic therapy which consisted of 300,000 units of penicillin every four hours and 0.5 gm. streptomycin every twelve hours. After the appearance of the ocular signs he was given 2 million units of penicillin intramuscularly every three hours and 500 mg. of achromycin® by the same route every six

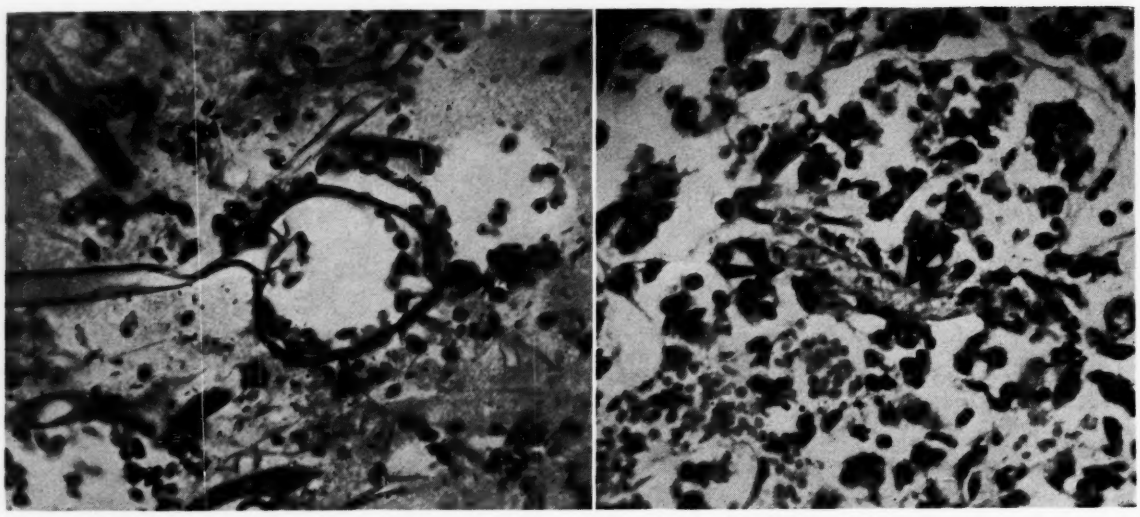

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FIG. 5. Mucosa of right ethmoid sinus. In addition to hyphae a sporangium attached to its sporangiophore and containing spores is shown. Scattered spores and many bacteria are present; Giemsa's stain; × 420.

FIG. 6. A single hypha is shown in the center (arrows) completely filling a capillary in the adenohypophysis; hematoxylin-phloxine; × 380.

hours. The patient became progressively stuporous and died on the sixth hospital day.

Autopsy findings revealed the following: The body was that of a well developed, slender Negro man. The principal gross findings involved the brain, the right orbit, the right frontal and both ethmoid sinuses and the intracranial portion of the right internal carotid artery. The dural sinuses were patent. The dura overlying the roof of the right orbit was roughened and adherent to the meninges of the frontal lobe. The mucosa of both ethmoid sinuses and of the right frontal sinus was dark red and necrotic, and necrosis of the underlying bone was noted. The left frontal sinus, the sphenoid sinus and the middle ears showed no gross abnormalities. When the bony roof of the right orbit was removed, the underlying periosteum bulged. On entering the retrobulbar space whitish, granular material was seen to envelop the eye muscles and to extend to the posterior aspect of the globe. A shaggy, greyish mural thrombus was present in the right internal carotid artery near the circle of Willis. In the mid-portion of the orbital surface of the right frontal lobe a 2 to 3 mm. area of softening was seen. Except for a slight cerebellar pressure cone, the rest of the brain showed no surface lesions. On sectioning, the area of necrosis at the base of the right frontal lobe extended into the adjoining white matter. Throughout the cortex and particularly in the floor of the sulci, many 0.5 to 1.2 cm. areas of discoloration were noted. They showed a red pinpoint center surrounded by a ring of greyish tissue with a pink halo. Similar lesions were present in the basal ganglia and cerebellum where they gave a distinct impression of surrounding a small blood vessel. The pons, medulla, spinal cord and pituitary revealed no gross lesions. Additional gross autopsy findings included several greyish areas of infarction involving the spleen and an accessory spleen, pulmonary congestion and edema.

Sections of the orbital surface of the right frontal lobe showed acute meningitis and extensive necrosis with marked infiltration by neutrophilic polymorphonuclear leukocytes and "gitter" cells. Numerous broad, branching, aseptate hyphae measuring from 100 to 200 μ in length and 7 to 15 μ in width were present. (Fig. 2.) These were found in the meninges and brain and also in the blood vessels where they filled the lumen and invaded all the layers of the wall. (Fig. 3.) Many vessels contained recent thrombi infiltrated with hyphae. The lesions scattered throughout the cerebrum and cerebellum consisted of recent infarcts with minimal inflammatory cell reaction at the periphery. Occasionally a small blood vessel containing mycelium was found in the center of the infarcts. In other areas the brain showed perivascular and perineuronal edema and oligodendroglial swelling. Sections taken through the right internal carotid artery at the site of the grossly

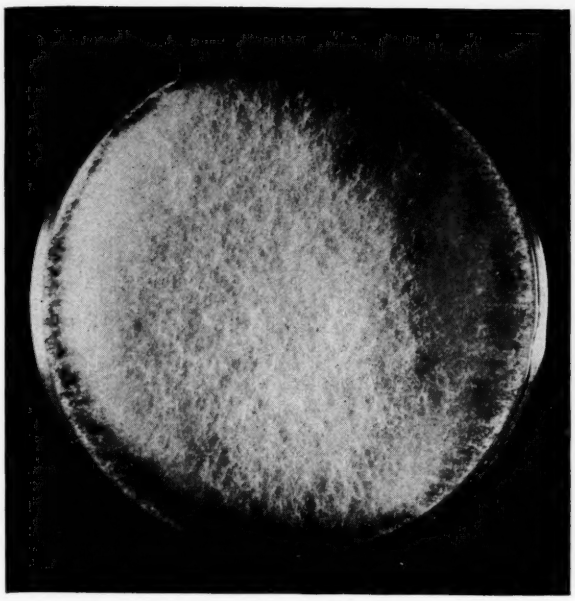


FIG. 7. One week old culture of Rhizopus oryzae growing on Sabouraud's (dextrose) agar.

described partial occlusion showed a recent mural thrombus teeming with hyphae which also infiltrated the vessel wall and perivascular sheath. (Fig. 4.) Sections of the dura from the right orbital fossa revealed areas of subacute inflammatory cell infiltration with some hyphae. Multiple sections representing the mucous membranes of the nose, nasal septum, right frontal and both ethmoid sinuses showed extensive necrosis of the tissue attended by an acute inflammatory cell infiltration varying from minimal to marked. In all these sections hyphae similar to those already described were found. They frequently invaded blood vessels. Also present in all sections were large numbers of microorganisms consisting of gram-positive and gram-negative rods and gram-positive cocci. Both hyphae and bacteria were seen to invade occasional spicules of necrotic bone and marrow. In the sections from the right ethmoid sinus many scattered spores and spore-containing sporangia were seen. Some of the sporangia were still attached to their sporangiophores. (Fig. 5.) Narrow, branching mycelium typical of actinomycetes were also encountered which differed from the already described fungus by the minute size of their mycelium and the lack of sporulation. These filaments averaged 1 μ in diameter and up to 50 μ in length. The orbital tissue showed invasion by the broad aseptate hyphae and by bacteria. A moderate degree of acute inflammatory infiltration was associ-

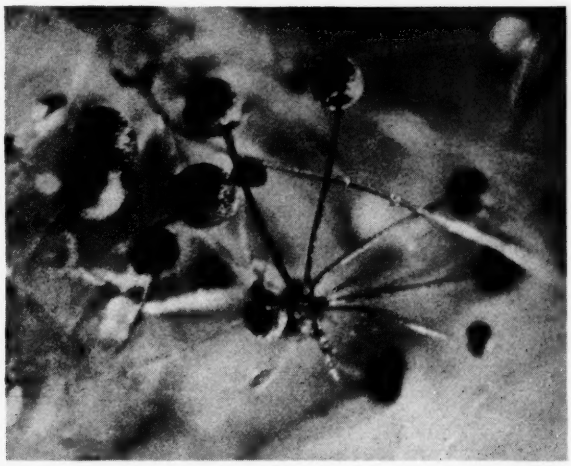
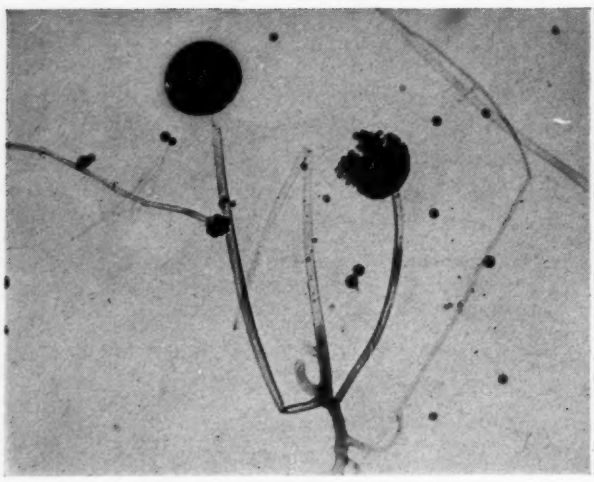


FIG. 8. Cluster of sporangiophores and sporangia of Rhizopus oryzae photographed by incident light; × 32.

ated with the lesions. The diaphragma sellae (turcicae) and the coverings of the pituitary stalk also contained many large, broad, aseptate hyphae, some of which appeared to lie within capillaries. The denser accumulations of the fungus were attended by a few neutrophilic polymorphonuclear leukocytes. Throughout the adjacent portion of the adenohypophysis were seen single hyphae without inflammatory cell reaction or necrosis of the neighboring pituicytes. (Fig. 6.) Additional histologic findings were slight glycogen infiltration of the liver and arteriolar nephrosclerosis. The splenic infarcts were surrounded by an area of fibrosis containing a medium-sized vein which was partly occluded by an endothelialized organizing thrombus. Rare renal tubules showed repair of the epithelium. The lungs revealed aspiration of foreign material including masses of bacteria and budding yeast cells typical of the genus Candida. The pancreas showed slight interstitial fibrosis.

Anatomic diagnoses: Diabetes mellitus (clinical); mucormycosis with meningo-encephalitis and involvement of the nasal mucosa, right frontal and both ethmoid sinuses, right orbital tissues, dura, right internal carotid artery and multiple recent infarcts of cerebrum and cerebellum; glycogen infiltration of the liver; interstitial fibrosis of pancreas; infarcts in spleen and accessory spleen; arteriolar nephrosclerosis, slight; acute tubular necrosis of kidney, slight, healing; pulmonary edema and congestion; aspiration of foreign material in lung.

Postmortem cultures were taken from heart blood, lung and from the right ethmoid sinus.



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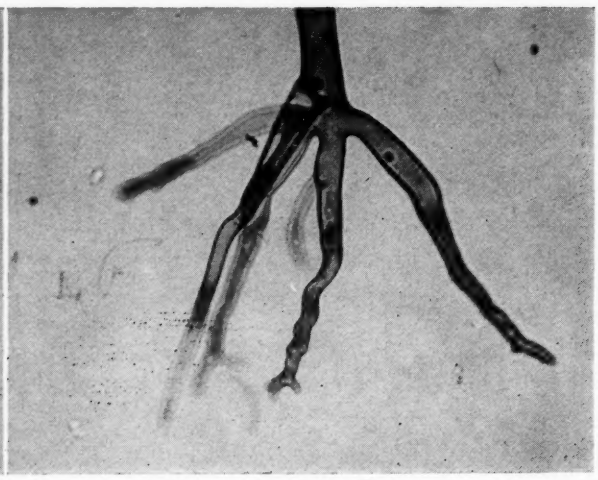

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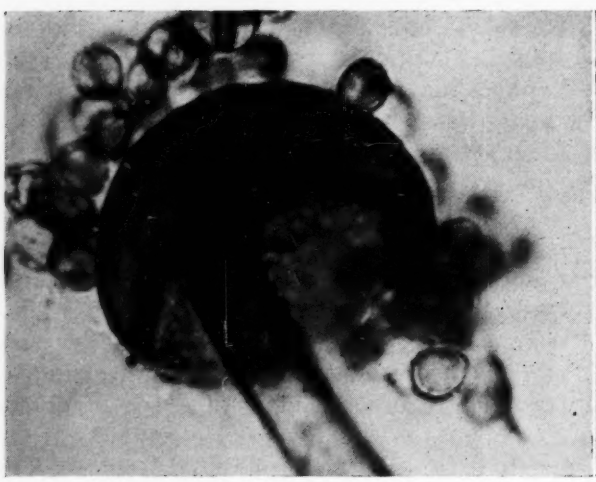
FIG. 9. Two sporangia and their sporangiophores subtended by rhizoids; × 200.

FIG. 10. Group of rhizoids characteristic of the genus Rhizopus; × 450.



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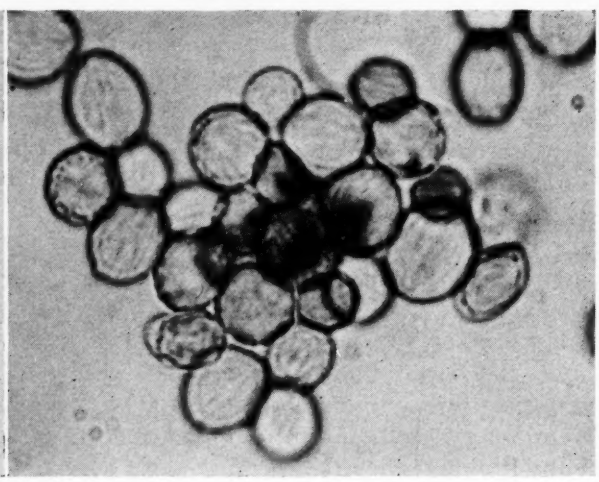

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FIG. 11. Columella of Rhizopus oryzae; × 980.

FIG. 12. Striated sporangiospores of Rhizopus oryzae; × 1200.

The heart blood showed no growth. The lung culture yielded a mixture of organisms consisting of enterococcus, Aerobacter aerogenes, a coagulase-negative hemolytic Staphylococcus aureus and possibly other bacteria which were not isolated. The results of the cultures from the right ethmoid sinus follow.

MYCOLOGIC STUDIES

Sabouraud-dextrose agar cultures of the material obtained from the right ethmoid sinus yielded a rapidly growing fungus that qui kly filled test tubes and plates with the greyish white mycelium typical of many species of the order Mucorales. (Fig. 7.) The loose mycelium darkened with age and assumed a yellowish brown color. At maturity, dark brown sporangial heads could be observed interspersed throughout the culture. (Fig. 8.) Microscopically, the colony was found to be composed of broad, aseptate, hyaline mycelium measuring 15 to 20 μ in diameter. Clusters of dematiaceous sporangiophores (325 to 700 μ long and 12 to 16 μ wide) (Fig. 9), subtended by yellowish-brown rhizoids (Fig. 10), arose along the aerial mycelium and bore large, spherical sporangia (100 to 160 μ in diameter) that were dark brown in color. The sporangia easily ruptured, releasing their spores and revealing a flat-domed hyaline columella (45 to 120 μ in diameter). (Fig. 11.) The light brown spores were striated and somewhat irregular in form and measured 6 to 8.5 μ in

length and 5 to 6 μ in width. (Fig. 12.) Zygospores were not observed. The fungus grew well at both 25°C. and 37°C. On the basis of these characteristics this phycomycete was identified as Rhizopus oryzae.[2] The identity of this organism was confirmed by Dr. W. C. Hesseltine, United States Department of Agriculture, Peoria, Illinois.

The salient features of the disease are summarized in Table I. From this survey of the literature and our own two cases there emerges a rather characteristic picture which should permit clinical diagnosis. All but four patients had proved or presumptive diabetes mellitus which was complicated in the majority of cases by acidosis. The typical sequence of events seems to consist of the appearance of ophthalmoplegia, usually unilateral, and evidence of meningoencephalitis in the presence of persistent hyperglycemia and glycosuria. In most instances the neurologic manifestations appeared after the diabetic acidosis had responded to therapy and the patients seemed clinically improved.

TABLE I

SUMMARY OF SALIENT CLINICAL AND AUTOPSY FINDINGS IN CASES OF CEREBRAL MUCORMYCOSIS

Authors	Age	Race	Sex	Tissues Involved					Diabetes Mellitus	Acidosis
				Orbit	Paranasal Sinuses	Cerebral Vessels	Brain	Other Organs		
Paltauf, 1885[3]	52	W	M	−	−	+	−	+	−	−
Gregory, Golden and Haymaker, 1943[4]	43	N	F	+	−	+	+	−	+	+
	52	N	F	+	+	+	+	−	+	+
	75	W	M	+	−	+	+	−	Questionable	Studies inadequate
LeCompte and Meissner, 1947[5]	57	W	M	+	−	+	+	−	Hemochromatosis	+
Wolf and Cowen, 1949[6]	42	N	M	−	+	+	+	−	+	−
Stratemeier, 1950[7]	32	N	F	+	+	+	+	−	+	+
Kurrein, 1954[8]	5 mo.	W	M	−	−	+	+	−	−	−
Martin et al., 1954[9]	2½ mo.	W	M	+	−	+	+	+	−	−
Case I	25	W	F	+	−	+	+	−	+	+
Case II	40	N	M	+	+	+	+	−	+	+

COMMENTS

In 1885 Paltauf[3] described a patient with a systemic fungus infection involving the central nervous system. On morphologic grounds he identified the agent as belonging to the order Mucorales and coined the term "Mycosis mucorina." There was no evidence of diabetes mellitus in this patient. No further reports of cerebral mucormycosis reached the literature until 1943 when Gregory, Golden and Haymaker[4] published their comprehensive review, adding three cases of their own. Five other patients with cerebral mucormycosis have since been described.[5–9]

To date, all patients with known cerebral mucormycosis have died and the diagnosis of the disease has been made only at autopsy. Postmortem findings reveal invasion by the fungus of the meninges, cerebral vessels, the brain and soft tissues of the orbits. In addition to meningitis, generally localized to the frontal

lobes, the brain shows multiple areas of infarction. These result from the invasion of blood vessels by the organism with formation of thrombi and emboli. The individual hyphae are often of such size as to occlude small blood vessels or capillaries. The inflammatory response to isolated hyphae is minimal and even clusters of mycelium are attended by little reaction. When appreciable inflammation is encountered it appears to be associated with tissue injury from vascular obstruction. Sinusitis may be another significant finding. In our second patient the mucous membrane of the right ethmoid sinus was the only site where sporulation of the fungus was encountered. There was also evidence of marked bacterial infection.

In previous reports the fungi have been assumed to be members of the genus Mucor despite the fact that phycomycetes cannot be identified by the appearance of their mycelium in stained tissue preparations. Definitive identification of the fungus by culture has been lacking. The etiologic agent in our histologic preparations resemlbes in every detail the appearance of the fungus described and depicted in previous reports. In our second patient the organism was isolated and proved to be Rhizopus oryzae, a species of the order Mucorales. This phycomycete belongs to a specialized group of fungi known as "sugar fungi."[10] As the name suggests, these fungi possess marked ability to break down sugar, and in nature these saprophytes are the first to colonize dead or dying plant and animal tissues. According to Garrett,[11] wide distribution in soil, a fast rate of mycelial growth and rapid spore germination enable these fungi to "flare up" whenever a suitable substrate is encountered. Although "sugar fungi" are normally saprophytic they are conceivably able to assume a parasitic state in carbohydrate-enriched tissues such as are found in uncontrolled diabetes mellitus. Under aerobic conditions and in the presence of a sugar-containing substrate the fungus readily sporulates, as seen in the sections from the ethmoid sinus of our second patient.

The occurrence of sinusitis has been described only in three previous cases when it was discovered at autopsy. Routine postmortem histologic examination of the paranasal sinuses in diabetics with suspected cerebral mucormycosis may reveal that this lesion is a common if not constant finding. Invasion by the fungus of the mucosa found in the ethmoid sinus of our second patient and extension of the process into the underlying tissue with involvement of the bone suggests that this lesion represents the portal of entry of the infection. These same sections also show severe acute bacterial infection of the tissues with associated acute inflammation. This may be a significant finding. Several factors may have to be present in order to permit the fungus to become pathogenic. The sustained hyperglycemia of poorly controlled diabetes mellitus is undoubtedly important. A further alteration of carbohydrate metabolism at the local level may be provided by the increased rate of glycolysis associated with acute inflammation. These factors as well as the presence of necrotic tissue perhaps produce a substrate suitable for the proliferation of the fungus and its invasion of tissue. The role of bacteria in this process is a matter for speculation.

The infection appears to spread by way of blood vessels. The striking capacity of the fungus to invade vessels has impressed previous observers of cerebral mycormycosis. We have no explanation for this behavior of the fungus. We are also puzzled by the rarity of this disease, considering the high incidence of diabetes mellitus and the ubiquitous presence of the Mucorales. Since at the present state of our knowledge the cerebral form of mucormycosis occurs only in some diabetics, one wonders whether these patients have an additional metabolic abnormality which permits these common saprophytes to become pathogenic. A review of the clinical and biochemical aspects of the diabetes in our patients and those previously reported reveals nothing which is not commonly encountered among diabetics in general.

Signs of retro-orbital infection, usually unilateral and culminating in ophthalmoplegia, as well as evidence of meningo-encephalitis in diabetic patients should suggest the possibility of cerebral mucormycosis. In most cases ocular signs are the earliest clinical manifestation. Histologic examination and particularly culture on Sabouraud's medium of scrapings and biopsies of the nasal mucous membranes may serve to corroborate the clinical impression. Culture of the cerebrospinal fluid for fungi should be performed. In diabetic patients it is advisable to regard the finding of large, broad, aseptate branching hyphae as clinically significant and not as contaminants.

Specific therapeutic agents for the treatment of cerebral mucormycosis are not available.

Bacterial infection may be a predisposing factor in the pathogenesis of the disease and antibiotics may therefore prove useful. Since prolonged hyperglycemia probably plays an important role in this fungus infection, rapid re-establishment and careful maintenance of a normal blood sugar level should be of therapeutic benefit. The adequate control of blood sugar levels attained with modern therapy in the majority of diabetic patients may provide a partial explanation of the rarity of cerebral mucormycosis.

SUMMARY

Rhizopus oryzae has been isolated and identified as the infectious agent in one of two diabetic patients dying of cerebral mucormycosis.

To our knowledge this rare and fatal complication of diabetes mellitus has never been diagnosed prior to the patient's death.

The clinical and autopsy findings of this fungus disease in our own and previously reported cases are reviewed. The paranasal sinuses are suggested as a possible portal of entry of the infection and problems of pathogenesis are discussed.

From this study emerges a syndrome consisting of uncontrolled diabetes mellitus associated with ophthalmoplegia, signs of meningoencephalitis and, possibly, sinusitis. These characteristic manifestations may permit an antemortem diagnosis of this disorder.

Acknowledgment: We are indebted to Dr. E. B. Agnor for permission to use his office records of the patient reported as Case 1.

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Homozygous Hemoglobin C Disease*

Report of a Case with Studies on the Pathophysiology and Neonatal Formation of Hemoglobin C

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IN 1949 Pauling and co-workers[1] made the observation that normal and sickle cell hemoglobin differ in electrophoretic mobility and therefore in their molecular structure. To the present, seven variations of the hemoglobin molecule have been described.[2–5] Workers in this field have agreed on a standard terminology for the various types of human hemoglobin.[6] Adult hemoglobin is designated hemoglobin "A"; sickle hemoglobin, "S"; fetal hemoglobin, "F"; subsequent abnormal hemoglobins are C, D, E and G.

Itano and Neel first described hemoglobin C.[2] Subsequent studies have delineated the clinical picture and hereditary pattern of hemoglobin C in combination with adult hemoglobin and in combination with sickle hemoglobin.[7] Sickle cell-hemoglobin C disease (CS) is characterized by a mild chronic anemia with erythrocyte sickling and target cells.[7,8] The combination of hemoglobin C with adult hemoglobin (AC or C trait) appears to produce no symptoms, the only abnormality being an increased number of target cells.

To date seven cases of homozygous hemoglobin C have been reported.[9–15] Spaet,[9] Levin,[10] Ranney[11] and Terry[12] and their respective associates have described the clinical picture associated with hemoglobin C disease. The following case presented the opportunity to make several studies outlining the pathophysiology of this disease and to add several new findings on hemoglobin C.

CASE REPORT

Mrs. G. K. (No. 3B7), a twenty-eight year old Negro, was admitted to the Peter Bent Brigham Hospital for the second time in January, 1954, for study of splenomegaly and anemia.

She was first seen in the outpatient department of this hospital in 1948 at the age of twenty-three. She gave a history of frequent colds, fatiguability, occasional headache and one hemoptysis two years previously. There was no previous history of serious illness. The patient had not had arthritic symptoms. The spleen was palpable, the hematocrit 34. Urinary sediment showed eight to ten red cells.

In August, 1949, she was seen again. The spleen was palpable, the hematocrit 36 and microscopic hematuria was again noted.

In October, 1949, she was admitted to this hospital for the first time for investigation of her anemia. Blood pressure was 125/80, the skin was normal and there was no lymphadenopathy. The lungs were clear and the heart normal. The edge of the liver could be felt on deep inspiration, and the spleen was palpable 2 fingerbreadths below the left costal margin. There was no clubbing of the fingers. Joints were normal. Neurologic examination was normal. Laboratory data at the time of this admission showed a normal urine except for five to six red blood cells in the sediment. The hematocrit was 35, the M.C.V. 94, M.C.H. 33, M.C.H.C. 36. Platelet count 180,000 white count 9,600 with a normal differential. BUN 7, total protein 6.1, fasting blood sugar 79, calcium, phosphorus, alkaline phosphatase, uric acid and thymol turbidity were normal. The bilirubin was 1.13 mg. per cent and later was 0.85 mg. per cent. BSP test showed 3 per cent retention at the end of thirty minutes. Sickling preparation was negative. EKG showed normal curves. The chest and bone x-rays were normal. A bone marrow aspiration was reported as normal.

In July, 1953, the patient was admitted to another hospital because of three episodes of

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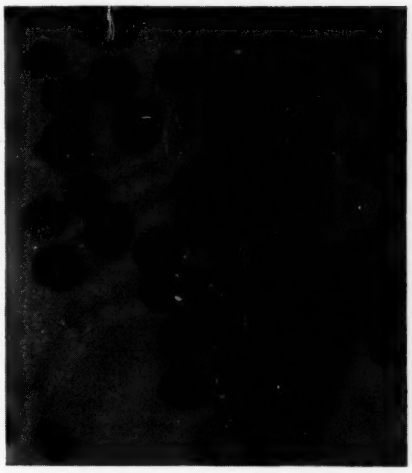
vaginal bleeding, the etiology of which could not be determined.

She returned to the outpatient department of this hospital in August, 1953, with symptoms of an upper respiratory infection and chronic fatigue. Chest x-ray was normal, hematocrit 26, M.C.V. 90, M.C.H. 33, M C.H.C. 26. The sickling preparation was normal. Because of the observation of numerous target cells in the peripheral smear a diagnosis of abnormal hemoglobin disease was made, paper electrophoresis was carried out and the patient was found to be homozygous for hemoglobin C.

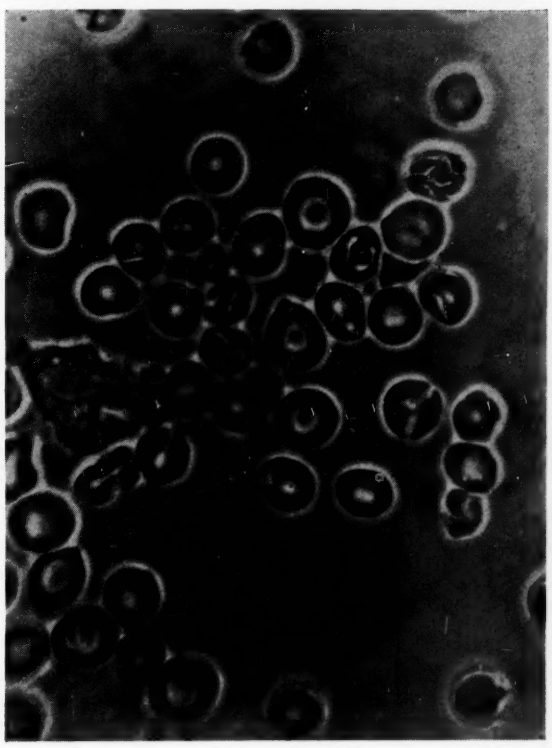
The second admission to this hospital in January, 1954, was for the purpose of carrying out the studies herein reported. The physical examination was unchanged, the spleen being 5 cm. below the left costal margin. The patient was noted to be pregnant and fetal heart sounds were heard. Laboratory data showed a normal urine analysis, hematocrit 24, reticulocytes 7 per cent; the peripheral blood smear showed 21 per cent target cells. (Fig. 1A.) Under the phase microscope the red cells had a striking appearance characterized by an irregular contour and folding of the cell membrane. (Fig. 1B.) The bleeding time was four minutes, clotting time nine minutes and the clot retraction was within normal limits. Platelet count 402,000, prothrombin time thirteen seconds. Pulmonary function studies were within normal limits. The arterial-alveolar gradient for oxygen was normal. X-ray studies showed the lungs, heart, bones and joints to be normal.

On April 5, 1954, at the Boston Lying-In Hospital, the patient was delivered of an apparently normal girl. Two [illegible]owing delivery the patient [illegible]atic; the hematocrit was 32 and the reticulocyte count 4 per cent.

The patient's parents were born in the West Indies. Both the mother and father are living and well. Two brothers and one sister died in infancy of an unknown cause. Four siblings are living and well but were not available for study. There is no known case of anemia in the family.



1A



1B

FIG. 1. A, smear of peripheral blood stained with Wright's stain. B, phase microscope picture of wet preparation of peripheral blood. (Courtesy of Dr. John Luft.)

SPECIAL STUDIES

Abnormal Hemoglobin Studies. Hemolysates of the patient's blood were examined by paper electrophoresis using a method previously described.[16] Figure 2 compares the patient's electrophoretic pattern with those of a hemoglobin C trait carrier (AC) and a normal individual (A). As in all other cases of homozygous hemoglobin C, this patient had a single abnormal component migrating with the mobility of hemoglobin C, a pattern diagnostic of the homozygous condition. In the heterozygous state (Figure 2) hemoglobin C is present in amounts of only 30 to 40 per cent while the remaining pigment is normal.[16,17]

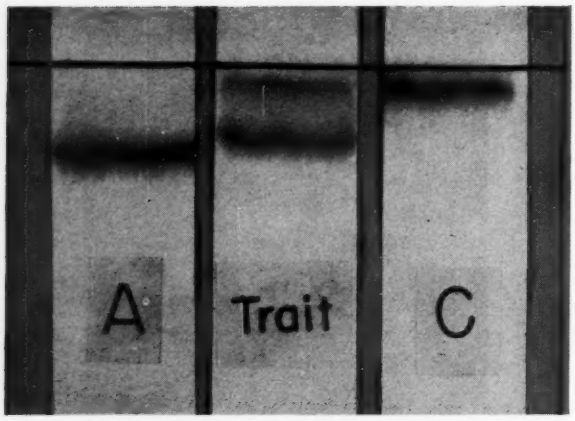


FIG. 2. A comparison of the electrophoretic pattern of the patient's hemoglobin (C) with that of a normal person (A) and a hemoglobin C trait carrier (Trait).

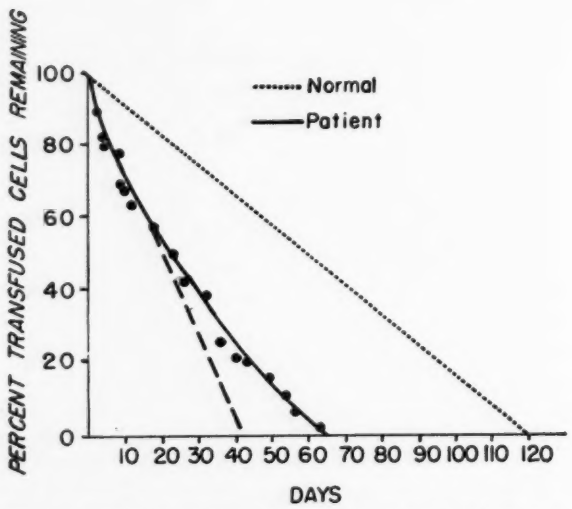


FIG. 3. Red cell survival time (Ashby technic) of this patient's cells transfused into a normal recipient.

The alkali denaturation test[18] revealed 2.4 per cent fetal hemoglobin. This value is abnormal since the normal range does not exceed 1.7 per cent.

Life Span Determinations. Five hundred ml. of the patient's blood were given to a normal recipient, and red cell survival was followed using the Ashby technic.[19] Figure 3 shows the results. The survival of the homozygous C cells was shortened. It should be noted that the survival time graph is of the exponential (concave) type with some red cells surviving to about sixty-four days. Using the device of extending the most rapidly falling slope of the survival time curve to the horizontal axis,[20] one arrives at a value for the mean red cell life span which is forty-two days. This finding agrees with the previously reported survival time studies with red cells from patients with homozygous hemoglobin C disease of fifty-five, forty-two and thirty days, respectively.[9,12,17]

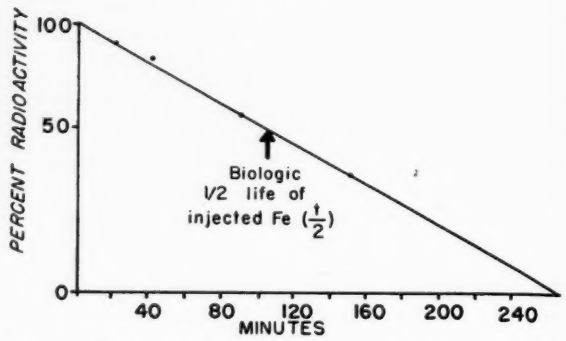


FIG. 4. The rate of disappearance of radioiron from the patient's serum.

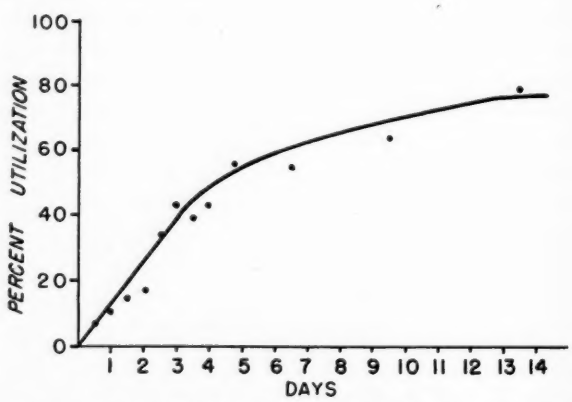


FIG. 5. The utilization of radioiron by the patient's red cells.

Radioactive Iron Studies. The serum iron was 87 gammas per 100 ml. (normal). The rate of disappearance of radioiron from the serum is shown in Figure 4. One-half of the injected dose was removed in 105 minutes (normal). The serum iron turnover[21] was calculated to be 33.9 mg./24 hr.* This value is within normal limits. The utilization of radioiron by red cells is shown in Figure 5. The value of 79 per cent in fourteen days is normal.[22]

Special Observations of C Hemoglobin. The solubility of this patient's hemoglobin was determined[23] and found to be 3.12 gm. per 100 ml. This is significantly higher than the solubility of normal hemoglobin (range 1.0 to 1.6 gm. per 100 ml.) and is considerably higher than that of

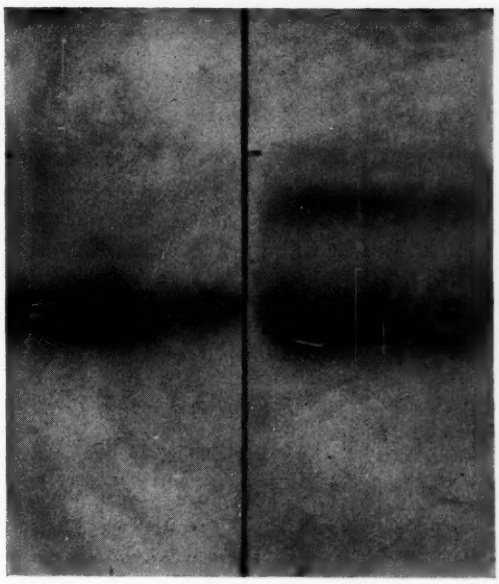
$$*\frac{0.693(k) \times 1{,}1440(\text{min./day}) \times 0.087(\text{serum Fe}) \times 4{,}100(\text{plasma vol.})}{105(\text{half-life of serum Fe}) \times 100.} = 33.9\ \text{mg./24 hr.}$$

sickling hemoglobin. These results agree with the findings of Itano, who found a significantly elevated solubility (3.46 gm./100 ml.) in one case of homozygous C.[23]

It has been shown that normal A hemoglobin forms refractile globules resembling Heinz bodies when mixed with sodium metabisulfite.[24] Sickling hemoglobin does not show this phenomenon. Red cells of this patient with homozygous C and of other individuals with C trait resembled A hemoglobin in that they formed Heinz bodies on treatment with sodium metabisulfite.

Fetal Hemoglobin vs. C Hemoglobin in the Newborn Period. The hemoglobin of this patient's husband was normal (AA). All offspring of homozygous C individuals with normal marital partners have been found to be C trait carriers (CA) (six children).[11,12,17] No other hemoglobin type could result from such matings on genetic grounds.[12] The birth of a child to our patient was awaited with great interest. Hemoglobin analysis of cord blood by paper electrophoresis and alkaline denaturation tests revealed 81 per cent fetal (F) and 19 per cent adult (A) hemoglobin. No C hemoglobin could be demonstrated. (Fig. 6A.) Four months later, paper electrophoresis of the infant's blood revealed the classic pattern of the C trait. (Fig. 6B.) F hemoglobin was 4 per cent at that time.

These results demonstrate that the gene for C hemoglobin, at least when present in the heterozygous state, expresses itself after birth. The production of F hemoglobin in fetal life thus has priority over formation of the later appearing, genetically determined C hemoglobin. Watson et al.[25] have made similar observations in sicklemia.



6A 6B

FIG. 6. A, the electrophoretic pattern of the cord blood. B, the electrophoretic pattern of the infant's blood at age four months.

COMMENTS

The studies of Terry et al.[12] have shown that the life span of normal red cells transfused into homozygous C patients is normal, indicating the absence of an extracorpuscular mechanism of cell destruction. Premature red cell destruction is therefore due to factors inherent in the erythrocytes.

An exponential type of survival time curve, such as found in this patient, is usually obtained when red cells from patients with hereditary hemolytic syndromes are transfused into normal recipients. Such a curve is interpreted as evidence of the random destruction of red cells. In hereditary hemolytic anemia a random hemolytic mechanism which destroys red cells regardless of age plus the accelerated senescence of the abnormally constituted cells is believed to explain the survival time curves. An alternate interpretation assumes no random hemolytic mechanism but postulates red cells of varying structure and composition which results in varying predetermined life spans. Proof for either theory is lacking in homozygous hemoglobin C disease. However, Singer and Fisher[26] have shown that multiple red cell populations with different life spans exist in sickle cell anemia.

The presence of an abnormal hemoglobin such as 100 per cent hemoglobin C in erythrocytes does not account satisfactorily for their premature destruction. Additional as yet unidentified chemical abnormalities of cell or stroma probably are present. At the state of present knowledge the basic hemolytic mechanism in this disease, as in most hemolytic anemias, remains unknown.

It has been previously pointed out[12] that the bone marrow of homozygous hemoglobin C patients is unable to compensate for the shortening of red cell survival to one-third of normal by raising hemoglobin production threefold. Since under certain circumstances the bone marrow may augment its hemoglobin output as much as seven or eight times,[27] failure to increase hemoglobin production significantly in this disease explains the anemia.

TABLE I

HOMOZYGOUS HEMOGLOBIN C CASES

Findings	References						Present Case
	Spaet et al.	Ranney et al.	Levin et al.	Kraus et al.	Terry, Motulsky and Rath	Rheingold and Holly	
Race	Negro	Negro	Negro	White (Italian)	Negro	Negro	Negro
Splenomegaly	Present	Present	Present	Present	Present	Present	Present
Anemia	Mild to absent	Mild	Mild	Mild	Mild	Mild	Mild
Type of anemia	Normochromic, normocytic	Normochromic, normocytic	Normochromic, normocytic	.....	Normochromic, normocytic	Normochromic, normocytic	Normochromic, normocytic
Reticulocytes	Normal	3–4%	Normal	90%	2–3%	.....	7%
Target cells	Numerous	Almost 100%	Moderate	28%	30%	Many	21%
Bilirubin	Normal	Mildly elevated	.....	Mildly elevated	Normal	Occasionally mildly elevated	Occasionally mildly elevated
Osmotic fragility	Decreased	Decreased	Decreased	Decreased	Decreased	Decreased	Decreased
Sickling	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Bone marrow	Normoblastic hyperplasia	Normoblastic hyperplasia	Normoblastic hyperplasia	Normoblastis hyperplasia	Normoblastic hyperplasia	.....	.....
Electrophoresis of Hb	100% Hb C	100% Hb C	100% Hb C	100% Hb C	100% Hb C	100% Hb C	100% HB C
Alkaline denaturation test	Less than 1% F Hb	Less than 1% F Hb	.....	.....	Less than 1% F Hb	.....	2.4% F Hb
Life span of normal RBC in patient	.....	.....	.....	.....	106 days	.....	.....
Mean life span of patient's RBC in normal	55 days	.....	30 days	.....	42 days	.....	42 days

Calculations of hemoglobin balance and determinations of radioiron turnover bore out the relative failure of hemoglobin production. The patient had a total circulating hemoglobin mass of approximately 470 gm. (Hb level of 9.4 gm. per cent times 5,000 ml. blood volume). To keep a steady hemoglobin level the patient destroyed and produced 11.2 gm. of hemoglobin per day (Hb mass of 470 gm. divided by mean life span of forty-two days). Normal hemoglobin production in a person of similar size is about 6.5 gm. per day. Only a mild increase (1.7 times) of normal hemoglobin production thus was present.

Considering the experimental errors involved in obtaining data for these indices of hemoglobin production, there is good agreement between the calculation of hemoglobin balance and the radioiron studies. This demonstrates failure of the bone marrow to produce sufficient hemoglobin to compensate for the hemolysis. The mechanism of the anemia therefore must be considered to be dyspoietic as well as hemolytic. Similar conclusions have been arrived at in sickle cell anemia and thalassemia.

The clinical and hematologic features of seven cases of homozygous hemoglobin C disease are summarized in Table I and will not be further discussed here. A discussion of the differential diagnosis of hemoglobin C disease is given in the paper by Terry, Motulsky and Rath.[12]

SUMMARY

The clinical features of a case of homozygous hemoglobin C disease have been described. This condition is characterized by a mild normochromic, normocytic anemia, many target cells, and a characteristic electrophoretic hemoglobin pattern. The mean red cell life span of the patient's cells in a normal recipient was found to be forty-two days. Iron metabolism studies demonstrated a normal serum iron and a normal rate of disappearance of radioiron from the serum. The twenty-four-hour serum iron turnover was normal. The shortened life span of the red cells plus the failure of the bone marrow to compensate for the hemolysis indicates that the anemia is both hemolytic and dyspoietic.

A child of this patient at birth had only fetal and some adult hemoglobin. Four months later the typical pattern of the C trait (AC) was present, demonstrating that hemoglobin C formation occurs only in the postnatal period.

Acknowledgment: The authors are indebted to Dr. John G. Gibson, II, for assistance in carrying out the red cell life span studies.

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The Apparent Activation of Salmonella Enteritis by Oxytetracycline*

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ONE of the most common complications of modern antibiotic therapy is the occurrence of "secondary infection" of the mouth and gastrointestinal tract by microorganisms ordinarily considered to be of low pathogenicity for these tissues of the human host.[1–3] The secondary invaders most commonly encountered have been Monilia[4] and gram-negative bacilli[5] in the oropharynx and staphylococci[6] in the esophagus and bowel. Such infections are assumed to be due to disturbances in the normal microflora of the mucous membranes resulting from antibiotic therapy.[7] Organisms insensitive to the antibiotic, but normally held in check by factors of bacterial antagonism,[8] may thus be allowed to grow luxuriantly and produce active infection. To date the only "secondary infections" reported have been caused by organisms normally present in small numbers in the human gastrointestinal tract. The following case report illustrates an instance in which a pathogenic species of Salmonella, apparently lurking in the bowel of a "carrier," became activated during a course of prophylactic chemotherapy.

CASE REPORT†

S. O., a fifty-three year old white business executive, was admitted to Barnes Hospital on January 15, 1954, for surgical repair of Dupuytren's contracture of his left hand. The contraction had been present for fifteen years and in recent months had produced moderate discomfort. A review of the patient's past history revealed that several years prior to admission to the hospital he had experienced a severe generalized dermal reaction following penicillin injections given for a pulmonary infection. When questioned, he also stated that he was sensitive to sulfonamide drugs. Forty years before he had had an appendectomy. He denied having had any recent gastrointestinal symptoms except for two or three mild and transient episodes of diarrhea during the past year; these he had attributed to "food poisoning."

Physical examination at the time of entry to the hospital revealed a well developed, well nourished male adult. The temperature was 36.8°C., pulse 80, respirations 16, and blood pressure 160/80. Positive physical findings included mild sclerotic changes in the retinal arteries, Dupuytren's contracture of the left hand and a healed abdominal scar in the right lower quadrant. The abdomen was soft and non-tender.

Laboratory data were as follows: Blood count: hemoglobin 16.1 gm.; white blood cell count, 7,700; differential count, 1 per cent basophils, 3 per cent eosinophils, 53 per cent segmented neutrophils, 35 per cent lymphocytes, 8 per cent monocytes. Urinalysis was normal and cardiolipin test negative.

On the day following admission an excision of Dupuytren's contraction was performed. Terramycin,® 250 mg. orally four times daily, was begun on the day of operation. On the third postoperative day the patient had a severe shaking chill and developed fever of 39.9°C., headache and lower abdominal discomfort. Physical examination revealed no abnormalities. The white blood cell count showed a leukocytosis of 11,750 with 1 per cent eosinophils, 76 per cent segmented neutrophils, 16 per cent lymphocytes and 4 per cent monocytes. Urinalysis was negative, and a blood culture was sterile. Roentgenograms of the chest suggested pneumonitis in the left lower lobe but a second film

† The authors are indebted to the patient's personal physician, Dr. Samuel B. Grant, and to his surgeon, Dr. Barrett Brown, for permission to report this case.

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taken on the following day was interpreted as showing only minimal discoid atelectasis in the left lower lobe. After thirty-six hours of treatment the terramycin was discontinued, and tetracycline, 250 mg. four times daily, was begun. On the fourth postoperative day the abdominal pain became localized in the right lower quadrant, and the patient began to have frequent loose stools which contained occult blood. The temperature rose to 40.4°C. Rigidity and tenderness were noted in the right lower quadrant. Because of the possibility that the fever and gastrointestinal symptoms were due to the tetracycline, this too was discontinued. Within twenty-four hours the abdominal pain had decreased, and after forty-eight hours the patient was afebrile. The diarrhea subsided rapidly (Fig. 1), and the stools became firmer in consistency.

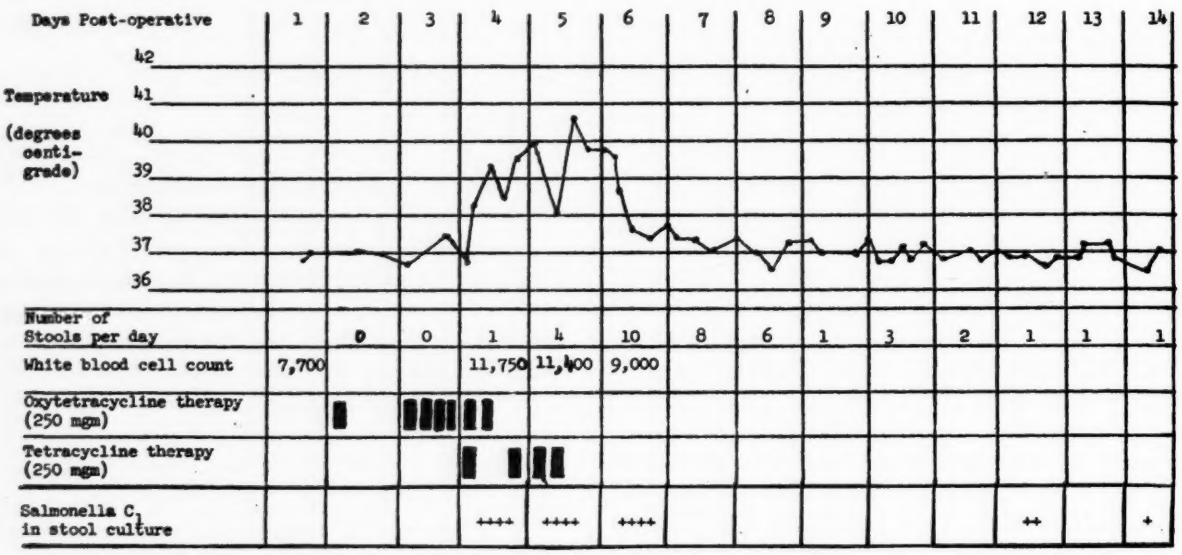

FIG. 1. Hospital course of patient developing Salmonella enteritis during postoperative antibiotic therapy.

Stool cultures were taken on the fourth, fifth and sixth postoperative days, and because of the possibility of the patient's having developed staphylococcal enteritis each specimen was inoculated onto both blood and desoxycholate agar. After twenty-four hours of incubation the culture revealed a heavy growth of a pure culture of Salmonella muenchen (group C_1).* The organism was found to be highly resistant to the tetracycline antibiotics, growing luxuriantly in broth containing concentrations of 50 μg./ml. To chloromycetin,® on the other hand, it was relatively sensitive, being inhibited by a concentration of 4 μg./ml. Similar cultures made on the twelfth and fourteenth day (one week after discontinuation of antibiotics) showed a return of the normal gastrointestinal flora and a marked decrease in the number of salmonella.

The patient was discharged after two weeks in the hospital and has had no further symptoms. Three follow-up cultures made six months later were negative for salmonella. No serologic tests were performed.

* Identified by the Missouri State Division of Health Laboratories, Jefferson City, Mo.

COMMENTS

Since this patient was studied in the hospital, experiments have been reported by Bohnhoff, Drake and Miller[9] which appear to have a direct bearing upon the phenomenon observed. Employing a streptomycin-resistant (but not streptomycin-dependent) strain of Salmonella enteritidis, they have clearly demonstrated that pretreatment of mice with large oral doses of streptomycin exerts a profound influence upon the microflora of the gastrointestinal tract. At the same time the antibiotic therapy makes the mice highly susceptible to orally induced infection with the salmonella strain, to which they are normally resistant. These observations, together with the extreme rarity of postoperative salmonella infection in man, make it only logical to attribute the activation of the salmonella enteritis in this case to the antibiotic therapy rather than to the operation.

The case history reported herein and the experiments of Bohnhoff et al. suggest that the use of the tetracycline antibiotics, to which

most strains of salmonella are relatively resistant, may not only be ineffective but also may even be dangerous when given to patients harboring salmonella organisms in their gastrointestinal tract.

SUMMARY

A case of acute gastroenteritis due to S. muenchen is reported in which the infection appeared to have been activated by prophylactic therapy with oxytetracycline. The probable mechanism of such activation is briefly discussed.

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An Unusual Case of Fatal Hepatic Sarcoidosis*

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INVOLVEMENT of the liver by sarcoidosis is not uncommon. Branson and Park in a recent extensive review of this subject[1] studied 1,106 liver biopsy reports and 138 autopsy reports from patients with sarcoid. Liver biopsy revealed lesions in 76 per cent of cases and there was 66.5 per cent involvement at post-mortem examination. Despite the frequent occurrence of hepatic lesions, jaundice is a rare manifestation of this disease. These same authors found only three cases, in addition to the one they report, in which icterus seemed definitely related to sarcoid of the liver. In a series of ninety cases at the Johns Hopkins Hospital jaundice was present in only two.[2] Wagoner, Freiman and Schiff report a case of sarcoidosis accompanied by jaundice in which granulomas were found by liver biopsy.[3]

Death as a direct result of hepatic sarcoidosis is even more rare.[1] This presentation deals with a patient who had extensive liver involvement accompanied by extreme jaundice. The patient was observed for one year, from the onset of symptoms to a fatal termination. During this time he was treated with ACTH, cortisone and x-ray without improvement. Transition from a granulomatous disease of the liver to one of severe fibrosis and scarring occurred.

CASE REPORT

S. S. (No. 352900), a forty-eight year old white, single, newspaper salesman, was admitted to Strong Memorial Hospital on October 31, 1952, with the chief complaint of jaundice. He had been in excellent health until one month prior to admission at which time he noted yellowness of the sclerae, as well as para-umbilical discomfort and nausea but no vomiting. There had been progressive increase of the jaundice and generalized itching for a month. Two weeks prior to admission he noted "bright yellow" urine for the first time and within the week preceding hospitalization there were loose "white" stools, general malaise and increased flatulence. There had been no chills or fever and no weight loss. His appetite had been good until two days prior to admission, and he had worked up to the time of hospitalization. On August 24, 1952, all of his teeth but two were extracted because of dental caries and pyorrhea. Novocain® injection anesthesia was used. There was no history of any other injections and no suggestion of exposure to, or ingestion of, hepatotoxins. He denied any alcoholic intake.

The past history was non-contributory. His only previous hospitalization had been sixteen years before because of lacerations of the thighs. He had been a newsboy for twenty years residing in Philadelphia, Seattle, and Rochester, New York.

Physical examination on admission showed him to be well developed, thin and deeply jaundiced. His temperature was 37.8°C.; pulse 80; respirations 20; blood pressure 108/60 mm. of Hg. No spider angiomas were present and no lymph nodes were palpable. The eyes were negative except for icteric sclerae. Examination of the heart and lungs was unremarkable. The abdomen was slightly protuberant, firm and rounded with no evidence of ascites. The liver was palpable three fingerbreadths below the umbilicus and was smooth, firm and slightly tender. The spleen, likewise, was remarkably enlarged and hard, the lower pole being felt two fingerbreadths below the umbilicus. Rectal examination revealed no abnormalities.

The hemoglobin measured 11.9 gm. per cent; hematocrit 39; corrected sedimentation rate 31 mm./hr. (Wintrobe). The white blood count was 3,000 and the differential showed 63 per cent

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neutrophils, 9 per cent band forms, 14 per cent lymphocytes, 5 per cent monocytes, 3 per cent eosinophils and 1 per cent basophils. Urinalysis was negative, except for a strongly positive bile test (Harrison spot test). The stool was light brown in color and negative for occult blood.

The serum bilirubin, alkaline phosphatase and total cholesterol were markedly elevated. (Table I.)

A blood Kahn test was negative as were brucella, typhoid, paratyphoid and heterophile agglutinations. Tuberculin skin tests (O.T.) were negative in 1:10,000 and 1:1000 dilutions and weakly positive with 1:100 dilution. A postero-anterior film of the chest was normal and x-rays of the hands and feet were also negative. An upper gastrointestinal series was negative, except for evidence of an enlarged liver and spleen. On November 5, 1952, a liver biopsy was done via the abdominal approach using a Vim-Silverman needle. On microscopic examination the tissue obtained showed granulomatous foci consisting of small whorls of pale-staining cells with fusiform or elliptic nuclei having the appearance of epithelioid cells. There was also evidence of chronic cholangitis but no fibrotic change. A sternal bone marrow biopsy revealed hyperplastic marrow. A section of the marrow showed one area suggesting a miliary granuloma. A splenic aspiration biopsy gave no results of pathologic significance.

The patient's icterus and laboratory findings showed no improvement and after considerable deliberation an exploratory laparotomy was performed on December 8, 1952, because of the possibility of a surgically remediable obstructive lesion. The liver was enlarged approximately one and a half times and appeared red, smooth and slightly swollen. There was no gross evidence of cirrhosis. The gallbladder was thin-walled, easily collapsible and contained one 6 by 8 mm. stone. The cystic and common ducts appeared normal and contained no stones. The spleen was four to five times enlarged and was firm. A large lymph node 5 cm. in diameter was present beneath the junction of the common duct and this was removed, as was a biopsy specimen from the left lobe of the liver. No free fluid was present in the abdomen.

Microscopic examination of the liver section showed conspicuous lymphocytic infiltration. In or adjacent to the portal areas were non-caseat-

TABLE I

Date	Bilirubin (mg. %)		Serum Proteins (gm. %)			Alk. Phos.	Cholesterol (mg. %)		T.T.	C.C.F.	P.T. (%)	Ca (mg. %)	P (mg. %)
	Direct	Indirect	Total	Albumin	Globulin		Total	Ester					
11/1/52	5.5	6.7	7.5	4.1	3.3	104	700	130	1.4	Neg.	100	...	...
12/9/52	6.8	6.8	7.5	4.0	3.5	132	715	197	0.5	Neg.	80	...	...
12/29/52	....	....	6.6	3.9	2.5	72	760	264	...	....	...	...	...
1/6/53	....	....	7.2	5.0	2.2	75	630	277	0.5	Neg.	65	...	...
2/4/53	....	....	6.2	4.2	2.0	76	785	192	1.0	Neg.	100	9.1	2.1
3/2/53	4.4	5.2	...	...	...	122	874	...	...	Neg.	...	...	...
7/21/53	25.6	23.4	5.8	1.0	4.8	184	...	...	...	2+	...	9.9	4.1
8/28/53	24	24.6	3.2	1.4	1.8	144	430	20	0.5	....	60	...	...
9/8/53	24.8	21.7	5.9	4.5	1.4	103	480	75	0.5	Neg.	...	...	...
9/21/53	16	14.4	4.3	2.9	1.4	100	575	85	...	2+	...	9.0	4.2

Cortisone and ACTH—December 25, 1952, through February 3, 1953, 2.825 gm. and 900 mg.
Cortisone—February 10, 1953, through March 10, 1953, 3.250 gm.
Cortisone—August 28, 1953, through September 25, 1953, 2.150 gm.

Alk. Phos.—Alkaline phosphatase—Bodansky units %.
T.T.—Thymol turbidity—Macglagen units.
C.C.F.—Cephalin cholesterol flocculation.
P.T.—Prothrombin time—% of normal.

* Protein fractionation by means of sodium sulfate.

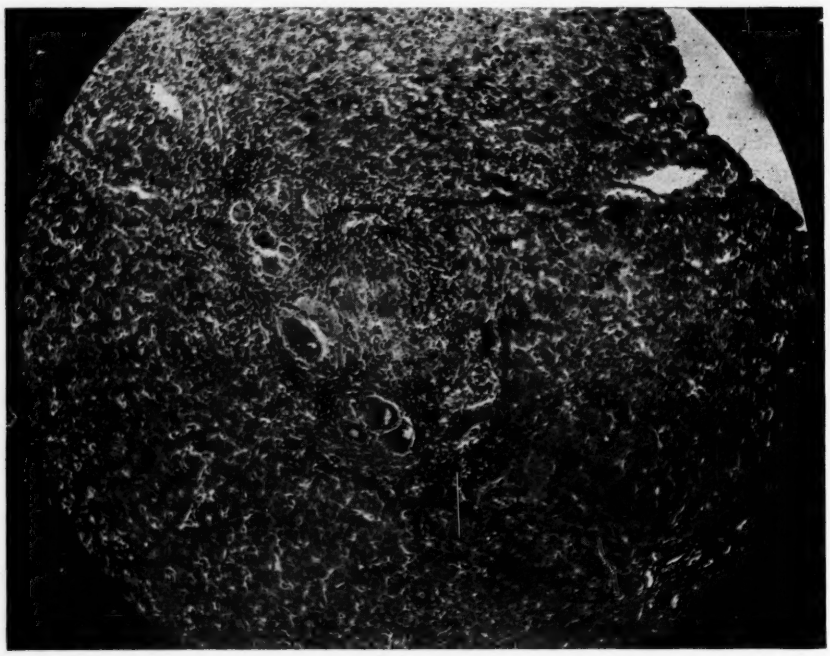

FIG. 1. Photomicrograph of section of liver removed at time of surgery, December 8, 1952, prior to cortisone therapy; hematoxylin and eosin, × 100.

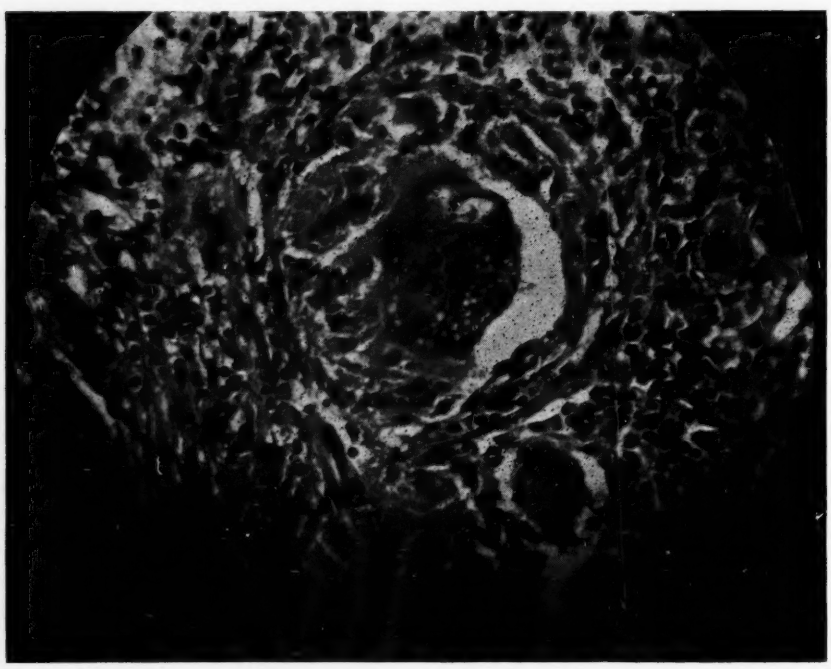

FIG. 2. Photomicrograph of giant cell with Schaumann inclusion body from section of liver, December 8, 1952; hematoxylin and eosin, × 430.

ing granulomatous foci of multinucleated giant cells with surrounding epthelioid cells, lymphocytes and a loose fibrous stroma. (Figs. 1 and 2.) The lymph node showed numerous multinucleated giant cells surrounded by epithelioid cells and a loose fibrous stroma. Within many of the giant cells were large purple- and yellow-staining particles, irregular and transparent, with dark irregular borders and a tendency to laminated structure. These bodies were similar to those described by Schaumann[4] and the entire histologic picture was consistent with sarcoidosis.[5] (Fig. 3.) Acid-fast preparations from the liver and lymph node were negative.

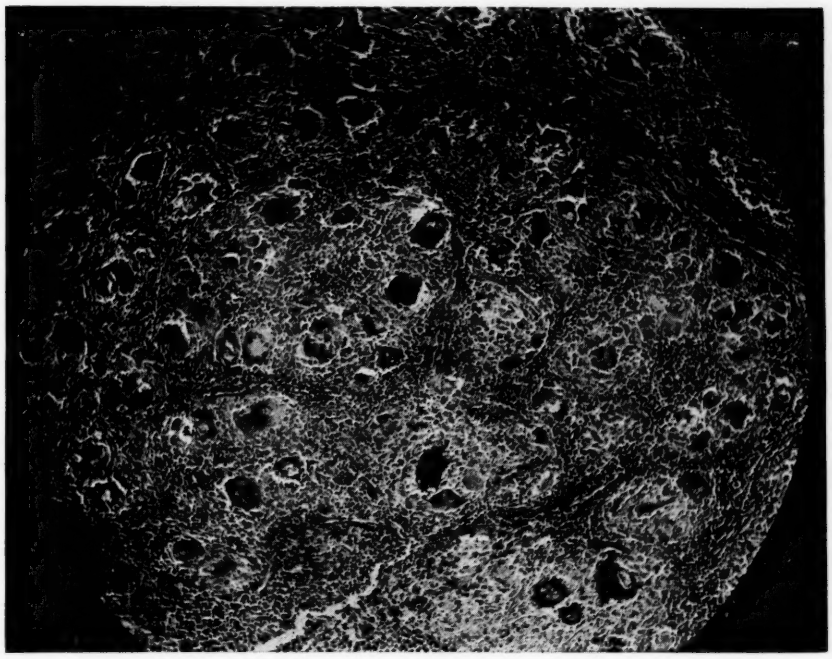

FIG. 3. Photomicrograph of section of lymph node removed at time of surgery, December 8, 1952; hematoxylin and eosin, × 100.

The postoperative course was complicated by bronchopneumonia, which responded to penicillin and streptomycin therapy.

On December 24, 1952, the patient was started on cortisone therapy, receiving 150 mg. by mouth daily for two weeks, and 100 mg. a day for one week. After the first week on cortisone there was some improvement in the blood findings, chiefly a fall in the alkaline phosphatase and bilirubin. (Table 1.) However, the biochemical improvement was not sustained and there was no evidence of clinical improvement. The patient developed fluid retention secondary to the cortisone therapy. Mercurials and restriction of salt caused a satisfactory diuresis.

A serum electrophoretic pattern by the Tiselius method was obtained on January 21, 1953. The protein values from this determination were as follows: total protein 9.4 gm. per cent, albumin 3.9 gm. per cent, alpha-1 globulin 0.6 gm. per cent, alpha-2 globulin 0.8 gm. per cent, beta globulin 1.6 gm. per cent, gamma globulin measured from the ascending instead of the descending boundary because of the so-called "beta disturbance" in the latter, 2.5 gm. per cent. (Fig. 4.)

On January 14, 1953, cortisone was discontinued. In the hope that ACTH would be more effective, 25 mg. daily by intravenous drip was started, the dose being slowly increased to 50 mg. On February 3rd an acute pneumonic process developed. ACTH was stopped and penicillin given. The pulmonary infection cleared promptly. Cortisone therapy, 200 mg. a day by mouth, was reinstituted on February

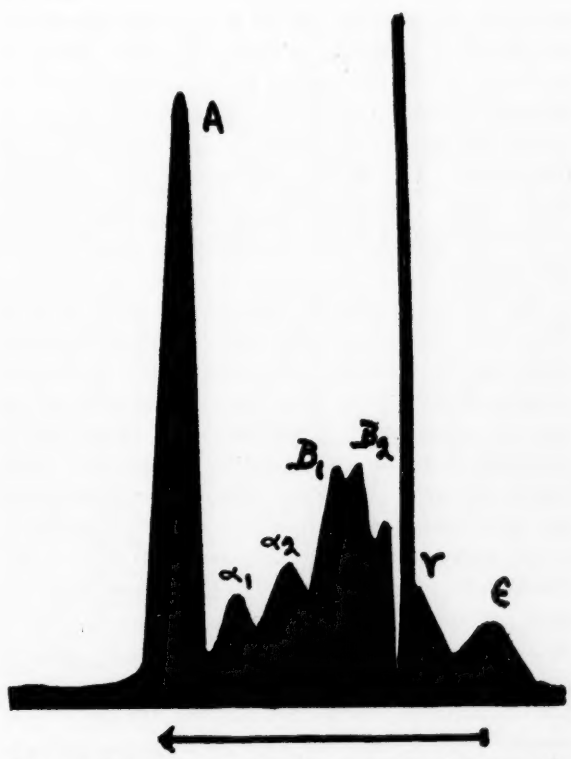

FIG. 4. Electrophoretic pattern, serum, January 21, 1953. Descending pattern showing "beta disturbance" appearing in the gamma peak.

10th. During this period the patient developed signs of adrenal hypercorticalism. Cortisone was gradually decreased and finally discontinued on March 10th. Cortisone therapy caused little if any change in the size of the liver or spleen and no evidence of clinical improvement.

Because of the lack of response a course of x-ray was thought to be worthy of trial. Favorable results have been observed at times following roentgen therapy.[6,7] This was begun on February 23, 1953. In the next two weeks a maximum of 2000r and a minimum of 1200r in daily increments of 150r was delivered to the liver via three portals. There was no decrease in liver size or relief of the jaundice. The patient was discharged from the hospital on March 16, 1953, with little improvement in his condition.

He was followed up in the medical outpatient department and shortly after discharge developed intense generalized pruritus associated with the persistent icterus. A second course of x-ray therapy was given and from April 29th through May 18, 1953, a tumor dose of 2200r in daily increments of 163r was administered to the liver without benefit. Massive doses of vitamin D, 50,000 units daily in the form of drisdol,® were started on June 9th because of favorable results reported in the Scandinavian literature.[8] The drug was continued through July with questionable benefit. Progressive weakness and debility necessitated readmission on August 27th.

Pertinent physical findings at this time, besides the deep icterus of the skin, were numerous excoriations and scratch marks and moderate edema of both feet. The liver was smaller than on the previous admission, the edge being palpable four fingerbreadths below the right costal margin. The lower pole of the spleen was felt three fingerbreadths above the left iliac crest.

He was again given cortisone, 100 mg. a day orally, and after two weeks of this therapy it was believed that there had been some improvement of the pruritus, less mental depression and a slight decrease in the size of the liver and spleen. On September 14th the patient became very weak, disoriented and appeared to be hallucinated at times. Cortisone was decreased to 50 mg. a day and was discontinued on September 25th. During this last admission occult blood was found in several stool specimens. A reduction of prothrombin time to 60 per cent of normal occurred. The hemoglobin measured 11.2 gm. per cent. An upper gastrointestinal x-ray series on September 21st showed coarsening of the rugal pattern of the stomach and duodenum but no evidence of ulcer. Parenteral vitamin K and general supportive measures were given but his course was one of progressive deterioration, and he died on October 13, 1953. The total dosage of cortisone he had received during the illness was 8.2 gm. and the total amount of ACTH was 900 mg. The total amount of radiation administered was a tumor dose of 4200r to the liver bed.

Autopsy Findings. Post mortem examination was obtained thirteen hours after death. The body was that of a well developed, poorly nourished, deeply icteric white male. There was no local or general lymphadenopathy. The abdomen was flat and the laparotomy scar well healed. The peritoneal cavity contained no fluid.

The liver weighed 2,100 gm. and the surface was smooth and of deep reddish color with a slight yellow mottling. The over-all consistency was finely granular with a decreased friability. On cut surface the lobular architecture was difficult to discern. It had a peculiar brownish red color against which minute tan areas could be seen. The pylorus and first portion of the duodenum were firmly adherent to the ventral surface of the liver at the level of the gallbladder, obscuring this organ. The gallbladder was entered with a probe and finger and noted to be patent, as were all the extrahepatic bile ducts. The wall of the gallbladder was thickened by adhesions. The extrahepatic bile ducts were of normal thickness with smooth mucosal linings. There was a single cholesterol stone in the gallbladder measuring 2 by 1 cm.

The spleen weighed 1,000 gm. Its entire surface was of a greenish cast. The cut surface appeared normal. The trabeculae and malpighian corpuscles stood out clearly against a deep purplish red parenchymal background. The stomach was markedly injected and contained a large amount of clotted blood. Six shallow ulcers were found in the antrum varying from 1 to 3 cm. in diameter. The duodenum, jejunum and ileum were filled with bloody material. The lymphoid tissue within the gastrointestinal tract was not remarkable but the periaortic nodes in the dorsal mesentery were enlarged, black and firm. The pancreas was of a greenish cast, the ducts were patent.

The left lung weighed 350 gm. and the right 450 gm. The pleural surfaces were smooth with a

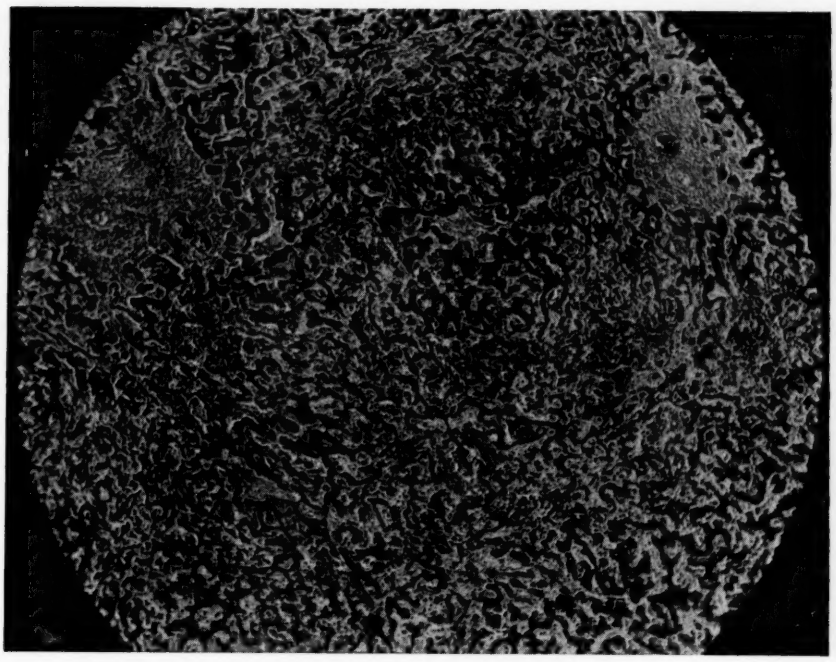

FIG. 5. Photomicrograph of section of liver (autopsy), October 13, 1953. Wright connective tissue stain, × 100.

yellowish cast. Small, firm and black hilar nodes were present. The bronchi were coated with bloody mucus. On cut surface the lungs varied from pink to red and were very wet. They were crepitant throughout with many emphysematous blebs. The heart weighed 300 gm. The endocardium was smooth with a yellowish tinge. The aortic valve showed a moderate degree of atheromatous plaquing, as did the coronary vessels and root of the aorta. The kidneys weighed 200 gm. each. Both were deeply bile-stained but of normal architecture. The adrenals measured 4 by 1½ cm. They appeared markedly atrophic with a very thin cortex and no distinguishable medulla. The bone narrow appeared grossly normal.

Microscopic examination of the liver showed extensive fibrosis throughout the parenchyma involving all zones, portal, mid-zone and central. There appeared to be a band of fibrous tissue surrounding almost every hepatic cord, extending from the central to the portal zones. It seemed most extensive and dense in the central areas and decreased slightly toward the periphery of the lobule, although it was still quite extensive in the portal areas. The hepatic cords were markedly compressed and atrophic in many areas. Many of the sinusoids were filled with large numbers of red blood cells. A considerable amount of dark brown pigment was present throughout the section, both within the Kupffer cells and the hepatic parenchymal cells. In the dense fibrous stroma of many portal areas were moderate numbers of round cells. (Fig. 5.)

In one of the lymph nodes taken from the dorsal mesentery there were multiple granulomas composed of clumps of hyalinized connective tissue, in and about which were large numbers of giant cells. Schaumann bodies and crystal inclusions were present within many of the giant cells. (Fig. 6.) No granulomas were found in the spleen. The capsule and trabeculae were thickened and partially hyalinized. The malpighian bodies were atrophic. Sections from the pylorus through the grossly visible ulcers showed ulceration of the epithelium with a base composed of large numbers of acute and chronic inflammatory cells infiltrated through the submucosa and musculature. There was marked capillary proliferation in the base of the ulcers.

Microscopic examination of the lungs showed an interstitial pneumonia. The heart was unremarkable except for a minimal increase in loose connective tissue in a few small focal areas. This was interstitial for the most part and not replacing myocardial fibers. There was a generalized deposition of bile pigment within the tubular cells of the kidneys, the greatest quantity being in the convoluted tubules. Bile casts were present in a large number of tubules. A moderate generalized increase in interstitial fibrous tissue was noted. The adrenal cortex was markedly

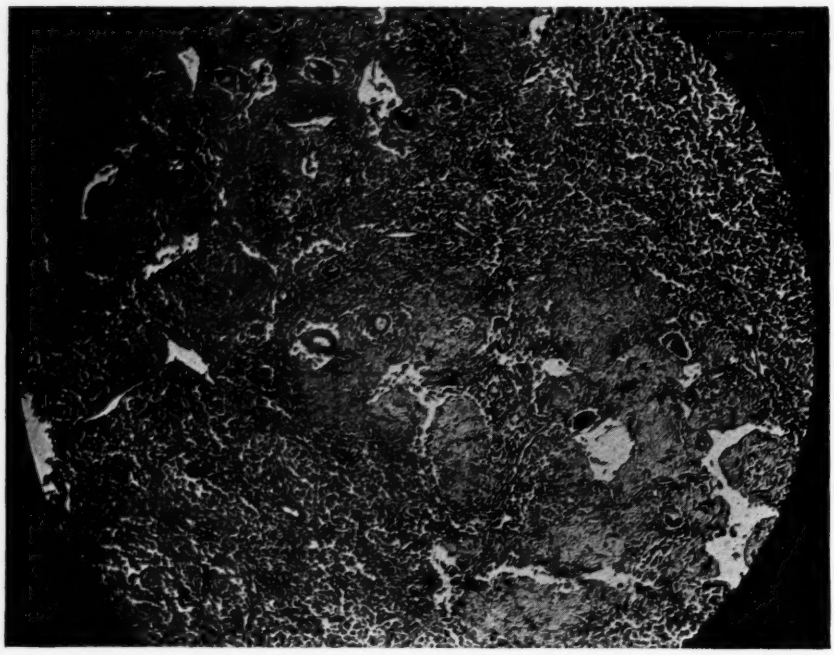

FIG. 6. Photomicrograph of section of lymph node from dorsal mesentery (autopsy) October 13, 1953; hematoxylin and eosin, × 100.

narrowed and the zones difficult to distinguish, particularly the fasciculata. In some areas there was no distinguishable reticularis. The medulla appeared normal in quantity and structure.

It should be noted that no sarcoid lesions were found in the bone marrow, adrenals, kidneys, gastrointestinal tract, spleen or lungs.

The final pathologic diagnoses were: multiple granulomas of liver and lymph nodes of dorsal mesentary suggestive of sarcoidosis, hepatic fibrosis, central, mid-zonal and portal, cholemic nephrosis, severe, atrophy of adrenal cortex, multiple gastric ulcers with massive gastrointestinal hemorrhage and interstitial pneumonia.

COMMENTS

One of the interesting and unusual features of this case is the presenting symptom of jaundice accompanied by hepatomegaly and splenomegaly. Sarcoidosis was not considered as a possible diagnosis until granulomas were found in the liver tissue obtained by needle biopsy. There seems little doubt that the patient's primary disease was hepatic sarcoidosis and that the resultant liver damage was the cause of his downhill course, unremitting jaundice and death. The blood chemical analyses gave evidence of an obstructive type of jaundice. Alkaline phosphatase levels were extremely high, a value of 184 Bodansky units per cent being obtained on one occasion. Serum cholesterol was also greatly elevated up to 874 mg. per cent. Despite the severe liver damage the cephalin cholesterol flocculation and thymol turbidity tests, which presumably reflect hepatocellular dysfunction, were consistently normal.

An unusual feature of the serum electrophoretic studies is the shift of the so-called "beta disturbance," usually present in the descending pattern, backward to the center of the gamma peak. This phenomenon has been encountered here in several cases of biliary obstruction in which the concentrations of plasma cholesterol and phospholipids were greatly elevated. In one of these cases in which the total lipids measured 2.0 gm. per cent, the electrophoretic pattern after extraction of the lipid at 0°C. with alcohol and ether showed a great reduction in the size of the beta and gamma peaks. In the case reported herein it seems probable that part of the increase in both beta and gamma peaks was due to their content of lipid.[9]

Regression of hepatomegaly and improvement in liver function tests have been noted in patients with sarcoidosis treated with ACTH.[10] In our patient, however, administration of cortisone and ACTH had no apparent beneficial effect in altering the course of the disease. One explanation for liver dysfunction in sarcoidosis is the pressure on and replacement of polygonal cells

by the granulomatous lesions. In addition, this same effect is exerted on the parenchyma and portal triads by the subsequent fibrosis and hyalinization which occurs in the course of the disease.[1] The possible benefit to be derived from cortisone therapy is that, if given early, the marked scarring which sometimes follows regression of the granulomatous lesions may be prevented.[11] The opposite effect seemed to develop in the patient described in this report. A severe degree of fibrotic change occurred in a ten-month period, as can be seen by comparing the liver sections obtained at the time of surgery with those from the autopsy. Such a rapid alteration seems unusual for the natural course of sarcoidosis. Indeed, the hepatic lesions of sarcoid may be quite benign and death due to liver involvement is rare. The patient of Branson and Park apparently had clinical manifestations of hepatic sarcoidosis for over two years before her death.[1]

Thus the rapidly developing cirrhosis in our case suggests that the administration of ACTH and cortisone may have produced a paradoxic therapeutic effect. The healing effect of the steroids on the sarcoid lesions possibly caused an accelerated change from a granulomatous picture to one of diffuse fibrosis and hyalinization. The resultant extensive scarring so damaged the parenchyma that hepatic insufficiency and death resulted. Such a therapeutic paradox is not unknown. Similar effects of accelerated healing have been noted following steroid therapy for sarcoidosis of the lungs and in cases of periarteritis nodosa. Patients with the latter disease studied at the Mayo Clinic by Baggenstoss and colleagues[12] showed initial improvement following cortisone administration but then died of cardiac and renal complications. All histologic signs of acute inflammation had disappeared at autopsy but the healing and fibrous obliteration of vascular lumina resulted in numerous infarcts particularly in the kidneys, heart and intestinal tract.

The possible role of radiation therapy in producing the fibrotic change in the liver must also be considered. Since the liver is highly resistant to radiation effect and the sarcoid lesion usually insensitive to x-rays in the dosage given here, it seems unlikely that it played a major role in causing the extensive scarring found at autopsy.

Recently it has been suggested that a vitamin D-like substance may be elaborated in sarcoidosis, which produces the elevated serum calcium sometimes found in this disease.[13] The patient reported herein was given large doses of vitamin D for over a month with no alteration in serum calcium. (Table I.)

SUMMARY

An unusual case of sarcoidosis, chiefly involving the liver is reported. In this patient severe impairment of hepatic function was produced by the granulomatous infiltration. Little or no benefit resulted from cortisone therapy and, indeed, there is evidence to support the opinion that steroid administration caused a paradoxic therapeutic effect by converting the granulomatous lesions to an extensive fibrosis of the liver.

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